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THE OHIO JOURNAL OF SCIENCE

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THE EFFECT OF ANTERIOR PITUITARY GROWTH HORMONE UPON THE COMPOSITION OF GROWTH.[1]

MILTON O. LEE AND NORWOOD K. SCHAFFER

The Memorial Foundation for Neuro-Endocrine Research, Harvard Medical School, Boston.

Certain extracts of the anterior pituitary body are now well known to produce unusual growth when administered to experimental animals and to human pituitary dwarfs. The physiologic or metabolic mechanisms through which these effects are produced are not yet known, however. In the experimental work described here the growth responses and the composition of the gains in weight were determined in rats treated with the hormone and maintained upon a limited food allowance.

Twelve groups of animals, each composed of litter-mates of the same sex, and initial body weight and length were used. One rat of each group became a control, a second received growth hormone and a third mate was killed and analyzed at the beginning of the experimental period. The control and its treated mate were fed daily the same amounts of food, according to the so-called "paired-feeding" technic developed by Mitchell.[2] The period of paired feeding and treatment ranged from eight to eleven weeks at the end of which the rats were killed by pairs and analyzed for water, fat, nitrogen and ash.

The anterior lobe extract used was prepared from fresh beef pituitary glands, purified and assayed. The amount injected daily was somewhat less than that necessary to cause a maximal growth response.

After treatment began the animals receiving the hormone immediately passed their control mates in weight and continued to show an excess gain each week until killed. The twelve

[1]Summary of paper presented before the Ohio Academy of Science, April 14, 1933, as part of a Symposium on Endocrinology.

[2]Mitchell, H. H. and J. R. Beadles. J. Nutrition, 2: 225. 1930.

controls weighed 2,382 grams initially and gained 764 grams; their twelve treated mates weighed 2,358 grams initially and gained 1,295 grams. The treated animals, on the same food consumption as their controls thus showed an excess gain of 531 grams, equivalent to the weight of two extra adult animals. Individually, eleven of the twelve treated rats showed an excess gain, a result which would be expected to occur by chance, only once in 340 times. The treated animals also showed significant excess gains in body length. By the technic of paired-feeding the amount of food given daily to the two members of any pair was determined by the appetite of the less hurgry member. In only 91 of 763 daily instances was a control rat more hungry than its treated mate.

The initial composition of both groups of animals estimated from the analyses of their litter mates, was: water, 62.0 per cent; fat, 12.7 per cent; nitrogen, 3.3 per cent; ash, 4.3 per cent; and energy, 2.33 calories per gram. The final composition of the controls was: water, 58.0 per cent; fat, 19.1 per cent; nitrogen, 3.0 per cent; ash, 4.0 per cent; energy, 2.83 calories per gram. The final composition of the treated animals was: water, 62.5 per cent; fat, 12.9 per cent; nitrogen, 3.2 per cent; ash, 4.1 per cent; energy, 2.32 calories per gram. The controls showed the characteristic changes in body composition with age that would be expected. These changes were statistically significant decreases in the proportions of water, nitrogen, ash and fat-ash-free dry tissue, and increases in the percentage of fat and in the heat value of the carcass. The treated animals, on the other hand, retained almost exactly their initial composition in all constituents and in the heat value of their tissues. Their gains in weight were consequently also closely similar to their carcass composition initially. The composition of the gains in weight, and of the excess gains by the treated rats is given in the accompanying table.

Statistical analysis of the data by Fisher's[3] measure of the significance of the difference in the means of small samples supports the inference that nitrogen and fat-ash-free dry tissue (protein) were the constituents most specifically affected by the hormone.

The nitrogen, ash and energy balances were determined in each group of animals, both directly from carcass analysis and

[3]Fisher, R. A. Statistical Methods for Research Workers. 3rd. Ed., London, 1930.

indirectly from the intake and excretion. The excess retention for the whole period by the treated rats over their controls was 22.5 grams of nitrogen and 22.4 grams of ash. The excess nitrogen retention by weeks closely paralleled the weekly excess gain in weight. The energy balance was slightly in favor of the controls, as they retained 341 calories (0.8 per cent of the total intake), more than their treated mates, despite the fact that their weight gain was some 500 grams less. The energy require-

TABLE I.

SUMMARY OF GAINS AND COMPOSITION OF GAINS IN CONTROL AND TREATED ANIMALS.

Group	Body Length Cm.	Live Body Weight Gm.	Empty Carcass Weight Gm.	Composition of Empty Carcass Gain Grams and Per Cent					Energy Calories
				Water	Ether Ext.	Fat-free Dry Tissue	Total N.	Ash	
CONTROLS—									
Total Gain	18.2	764	716	324	281	111	15.4	22.7	3161
Percent of Gain in Empty Carcass Weight				45.2	39.3	15.5	2.15	3.16	4.41*
Percent of Initial Datum	7.4	32.1	31.3	22.8	96.7	19.2	20.7	23.1	59.3
TREATED—									
Total Gain	33.1	1295	1217	771	162	284	37.9	45.1	2820
Percent of Gain in Empty Carcass Weight				63.3	13.3	23.4	3.12	3.71	2.32*
Percent of Initial Datum	13.5	54.9	53.7	54.9	56.3	49.6	51.5	46.4	53.5
EXCESS GAIN OF TREATED	14.9	531	501	447	—119	173	22.5	22.4	—341
Percent of Excess Empty Carcass Weight				89.2	—23.8	34.5	4.49	4.47	—0.68*

Empty carcass is the carcass minus the contents of the alimentary tract.
*Calories per gram of empty carcass weight.

ments for muscular activity were somewhat greater in the treated, but their energy expenditure per gram of weight gained was considerably less than that of the controls.

It is concluded that although the amount of the food consumption may be a limiting factor for growth under certain conditions, the growth hormone through its influence upon metabolic processes, is able to affect growth independently of the food intake. The growth produced has the same characteristics as the growth exhibited by young animals—a marked retention of nitrogen, synthesis and deposition of protein and a relatively high content of water and low content of fat.

FUNCTIONS OF THE ADRENAL CORTEX.[1]

FRANK A. HARTMAN
Department of Physiology, University of Buffalo.

Recent work carried out in our own and other laboratories has indicated certain functions of the adrenal cortex. We propose to briefly review some of these functions.

It has been known for over three-quarters of a century that the adrenal glands are essential for life; but not until 1916 did Wheeler and Vincent demonstrate that it was the cortex of these glands which was so vital.

ADDISON'S DISEASE.

The first really important contribution to the understanding of the function of the adrenal was the work of Thomas Addison, an English physician, who described the effects of disease in these organs. We now know that an absence of certain functions on the part of the cortex is responsible for these changes. Let us examine the picture from the earliest symptoms to the extreme prostration in late disease.

Asthenia.—Asthenia is the first to appear. This is so insidious that the patient may be unable to date its first appearance. He recognizes it only as an inability to perform accustomed tasks without fatigue, a condition which might be accounted for in many ways. Therefore, until the disease becomes farther advanced, it is usually ignored.

The asthenia involves the nervous system as indicated by the easy fatigue of the mental processes and the intolerance to stimuli. In the later stages general debility reduces activity to a minimum and a profound inertia develops, finally ending in prostration or coma. Other evidences of the involvement of the nervous system are insomnia, mental depression, failure of memory, poor judgment, lack of co-operation. Later there may be heightened motor activity and finally twitching and convulsions. If one administers an extract containing cortin, the vital hormone of the adrenal cortex, a patient in the late stages of Addison's disease responds with a disappearance of

[1]Presented before the Ohio Academy of Science, April 14, 1933, as part of a Symposium on Endocrinology.

the symptoms in the reverse order. Coma is replaced by increased motor activity, twitching and marked myotatic response to stimuli. With the return of consciousness, there may be disorientation, mental irritability and lack of co-operation. Later a state of calm is reached in which the heightened irritability disappears, pain is relieved and the patient becomes more rational and sleeps much. Finally the stage of recovery is reached in which mental alertness returns and asthenia is reduced. The patient sleeps well and not abnormally long as before. He becomes co-operative and takes an interest in his surroundings. These improvements begin to appear usually within a few hours after injections start, and recovery is reached within two or three days.

Experiments with animals have proven conclusively the widespread involvement of the nervous system in the asthenia of cortin insufficiency. By using a reflex preparation of the rat, we have been able to show that cortin will prevent to a large extent the fatigue which develops after the removal of the adrenal glands. This fatigue or asthenia is found in the reflexes, the myoneural junction and the muscle.

The subjective evidence from patients, as already stated, indicated that cortin played a role in the function of the higher centers. Anderson, Liddell and Hartman have shown objectively that cortin can influence the higher centers. They found that sheep made neurotic by attempting to develop a fine discrimination in the conditioned reflexes were improved by the injection of cortin. Under its influence, spontaneous movement almost disappeared while the response to the conditioned stimulus more nearly approached the magnitude of that in normal sheep.

Hartman, Beck and Thorn have shown that cortin has a pharmacological effect on the nervous system. Cases in which fatigue was the outstanding complaint have sometimes shown improvement in the nervous symptoms with cortin injections. Mental irritability was decreased, sleep improved and resistance to fatigue, both mental and muscular, was increased.

Cortin affects the nervous system in a normal individual under certain conditions; namely, when he is below par from over-work or from an infection. Improvement may be reflected in better sleep, and an increased sense of well-being sometimes to the point of euphoria. There is no proof, however, that in these subjects there was a cortin insufficiency. It might be

explained as a pharmacological action—merely the effect of added cortin to that normally poured into the blood stream.

As already stated, muscle as well as the nervous system is involved in the asthenia of cortical insufficiency. A number of workers have observed that the metabolic activity of the muscle itself is greatly reduced.

Asthenia also involves the circulatory system. According to many authorities, however, this is not the first system to be affected. In the later stages the heart action may be feeble. This may be similar in origin to the weakness found in muscle. The blood vessels themselves may be at fault. The poor compensation to change in posture indicates as much. A subject in advanced Addison's disease not only may have a blood pressure lower than normal but when he changes from a reclining to a sitting or standing position the pressure may fall. This may be due to failure of the reflex mechanism. In the later stages, the fluid may be lost to the tissues and the blood may become concentrated. A number of workers as far back as 1927 have suggested that the function of the adrenal was to regulate the proportion of plasma. Viale and Bruno attributed the increase of permeability to the nervous system and to changes in the composition of the blood. We have found that removal of the adrenals causes an increase in the water content of many of the tissues, such as skin and liver. When adrenalectomized animals are exposed to heat, water shifts less readily from the reservoirs into the blood. Such animals suffer from heat more readily than animals treated with cortin.

Kidney.—The kidney becomes involved in adrenal insufficiency. In the late stages particularly this is true, and it seems to appear before the blood pressure is sufficiently low to account for the reduced function. In late stages of adrenal insufficiency the blood urea may become quite high.

Gastrointestinal instability.—Gastrointestinal instability is an outstanding symptom of Addison's disease. In some patients, it may occur quite early; in others, later. This may be accounted for in part by changes in the nervous system. In the later stages the circulation may be a factor. Not only the more sluggish circulation associated with hypotension but hemorrhages and ulcers which develop indicate as much. Cortin stops the nausea and vomiting, and brings about recovery of appetite. It likewise stops the hemorrhages which sometimes occur from the alimentary canal in late insufficiency in animals.

You perhaps begin to realize that the vital hormone seems to be essential for all the tissues.

Metabolism.—It was shown in 1922 by Aub, Forman, and Bright that removal of both adrenals in an animal reduced the metabolism. In 1928 we were able to show that metabolism could be maintained within normal limits with small amounts of cortin. On the other hand, it has been impossible to raise the metabolism above normal either in animals or in normal human beings by the injection of large amounts of cortin.

Effect of cold.—The need for cortin under stress is very well demonstrated on exposure to cold. Adrenalectomized rats divided into two groups, one injected with salt solution and the other with cortin, at first show no difference on exposure to cold, but very soon the animals without cortin begin to be seriously effected. A study of the heat production has shown us that although both those treated with cortin and those only injected with saline at first produce the extra heat required, later those without cortin are not only unable to produce the extra heat required, but they produce less than they did normally at ordinary temperatures. Thus, we find a marked fall in their body temperature and some of them even die. The failure to produce the heat may be accounted for by the fatigue of the reflexes which are involved in response to cold, and the failure of the muscle itself to carry on the increased activity in the long run.

Growth.—Cortin is essential for growth whether it be the natural development of young animals or the renewal of tissue in the healing of wounds. In adrenal insufficiency growth or the healing of wounds may stop. Upon the administration of adequate amounts of cortin, growth is resumed.

Resistance to toxins.—In adrenal insufficiency there is a lowered resistance to various toxins. That resistance can be raised by the injection of cortin.

RELATION TO VITAMINS.

Cortin appears to bear some relation to the utilization of vitamins B_1 and C. The use of extracts containing cortin was suggested by the well-known changes which occur in the cortex of the adrenal gland in deficiencies of these vitamins. These changes indicated a possibility of extra demand on the gland. When an extract containing cortin was given to guinea pigs on vitamin C deficient diets the onset of scurvy was delayed.

The extract was prepared in such a way that none of the vitamin which itself prevents scurvy could have been present. Injection of this extract also delayed the onset of nervous symptoms due to a deficiency of vitamin B_1 in the diet.

CHEMICAL CHANGES.

The various chemical changes which appear in adrenal insufficiency have been followed by different investigators with an attempt to find a change which is a key to the disturbance in function without success. The evidence seems to accumulate that cortin is a general tissue hormone.

CORTILACTIN.

We have been able to separate from cortical extract a substance which is necessary for lactation. Adrenalectomized mother rats treated with amounts of cortin much more adequate than that required to maintain weight and health does not enable them to raise litters of young. A second substance must be added. Young rats nursed by adrenalectomized mothers receiving only cortin begin to die off a few days after birth, with 10 per cent or less reaching the stage of weaning. Adrenalectomized mothers, however, furnished with this new hormone in addition to cortin are able to raise a considerable portion of their litters. This new hormone which is essential for lactation has been named cortilactin.

We may say in conclusion that there is now positive evidence that the adrenal cortex produces two hormones: one, cortin, the vital hormone which seems to serve as a general tissue hormone; and, two, cortilactin, which is necessary for milk production.

Psychology, Genetics, and Intelligence.

This volume is an attempt to gather within a single cover the voluminous material on mental testing, and the relation of intelligence to its twin causes, heredity and environment. The book appears to be a very complete summary of the literature, including a considerable number of references with which the casual student would be unfamiliar, and some which even the specialist might readily have missed. The most valuable inclusions in the book, however, are the critical comments on the abstracted material, which are keenly analytical and are constructively presented. No more timely book in the field of human biology could well be imagined.—L. H. S.

Heredity and Environment, by Gladys C. Schwesinger. ix+484 pp. New York, The Macmillan Company, 1933.

THE DISTRIBUTION OF CUTIN IN THE OUTER EPIDERMAL WALL OF CLIVIA NOBILIS.

DONALD B. ANDERSON
University of North Carolina, Raleigh, N. C.

Epidermal cell walls are commonly considered by botanists as cellulose membranes upon which more or less cutin has been deposited. On the evidence of earlier microchemical work three general regions or zones in epidermal walls are recognized: a thin outer layer of cutin (the cuticle), an inner zone of cellulose and an intermediate zone containing both cutin and cellulose.

The inadequacy of this conception has been demonstrated in brilliant fashion by Frey-Wyssling (2). By studying the effect of the wall upon polarized light Frey-Wyssling has revealed a complexity of structure in certain epidermal walls that was entirely unsuspected. The outer epidermal walls of petioles of *Acuba japonica* were shown to possess curiously isolated plates of cutinized cellulose over each cell. The outer epidermal wall of leaves of *Clivia nobilis* were shown to possess marked differences in double refraction at different levels and a definite zone of isotropic material near the center of the wall, (Fig. 1A).

These researches of Frey led to a further examination of the detailed structure of these epidermal walls from a microchemical point of view. In the case of *Clivia*, the writer (1) was able to detect the presence of three membrane constituents in the wall—cellulose, cutin and pectic materials. The structural relationships between these three membrane constituents is outlined in Fig. 1B. The decrease of double refraction in the cellulose layer Z was related to an increase in amount of colloidal pectic material. The isotropic zone P was caused by the dominance of colloidal pectic material which reached its maximum in this zone. The rapid increase in double refraction as the outer portions of the wall were approached was explained by the decrease in abundance of pectic material in the wall. The maximum double refraction was reached at about the center of the zone of cutinized cellulose and at this point the pectic material reached a minimum. The decrease in double refraction as the cuticle was approached resulted from the steady decrease in the amount of cellulose present until finally when the cuticle C was reached cellulose was no longer present and the wall

became isotropic. The results of the microchemical investigations of this wall suggested an interpretation of the optical results. The two lines of investigation supplemented each other nicely and the results suggest the importance of using both lines of attack in cell wall work.

During the past summer an interesting modification of this epidermal wall was discovered in the leaves of a plant (Clivia) growing in the greenhouse of the Department of Botany of

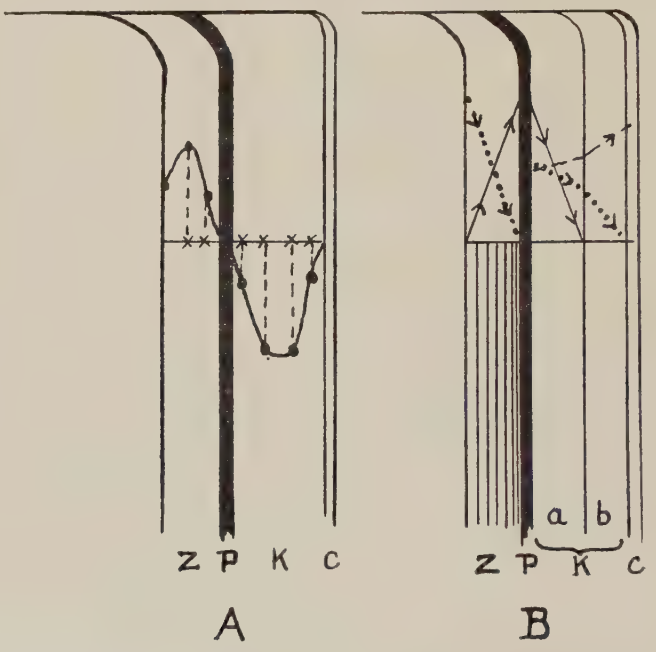

FIG. 1. Diagrammatic representation of the birefringence and chemical relationships in the outer epidermal wall of *Clivia nobilis*.

A. Diagram of the variation in double refraction at different levels in the wall (after Frey). Z represents the cellulose portion adjacent to the cell lumen. Its double refraction reaches a maximum at the center of the layer and drops rapidly as the isotropic zone P is approached. K represents the cutinized cellulose and is optically negative. Double refraction reaches a maximum at the center of the zone and falls rapidly as the outermost layer, C, the cuticle, is approached.

B. Diagram of the relationships of cellulose, pectic material and cutin in the same wall (after Anderson). The cellulose layer Z contains both pectic material and cellulose, the former increasing in amount as the isotropic zone is approached and the latter decreasing. The isotropic zone P contains pectic material with little or no cellulose. The cutinized cellulose K is divided into two zones, one of which (a) contains cellulose, pectic materials and cutin. Pectic materials decrease in amount, cutin increases in amount and cellulose decreases slightly as the zone (b) is approached. Zone (b) contains only cutin and cellulose with the latter decreasing in abundance as the cuticle is approached. The cuticle, C, contains cutin only.

The Ohio State University. Certain leaves of the plant showed a remarkable secondary cutinization of the inner portion of the wall that is apparently unique in epidermal walls. This unusual development is not characteristic of Clivia plants in general yet since it is not only an interesting phenomenon but one that may have an important bearing upon ideas regarding the mechanism of cutinization, the wall was given further study. This paper reports the results of a microchemical investigation of this epidermal cell wall.

METHODS.

Living leaves from the Clivia plant were cut with a sliding microtome into sections 8 or 10 microns in thickness. Similar sections were prepared from leaves preserved in 95% alcohol. These sections were subjected to the usual microchemical tests for membrane substances. Tests of three types were employed, (a) differential staining reactions, (b) differential solubility reactions, and (c) polarized light. The polarizing microscope alone permits the use of three different methods of determining the distribution of certain membrane constituents. Some membrane substances exhibit double refraction while others are isotropic (Plate I, Fig. 7). Insertion of a gypsum plate Red 1 between the nicols permits the use of interference colors to distinguish between some membrane compounds (Plate I, Fig. 8). Advantage may also be taken of dichroism in studing the distribution of lignin or cutin in cellulose membranes.

The various methods serve to check each other. Differential staining is not thoroughly reliable in all cases but is a valuable indicator of the presence or absence of specific materials. Results were not considered conclusive unless indicated independently by each line of attack. The following table gives a brief summary of the principal tests used in this study:

CELLULOSE.

Deep blue color with I_2KI and 65% H_2SO_4.
Blue violet color with chlorzinc iodide.
Unstained with methyl green unless combined with cutin and pectic compounds.
Unstained with Magdala red (echt) unless combined with cutin and pectic compounds.
Unstained with Ruthenium red unless combined with pectic materials.
Soluble in copper oxide ammonia.
Soluble in 50% chromic acid.

Insoluble in hot dilute mineral acids.
Optically positive.
Optically anisotropic.

Pectic Compounds.
Deep red color with dilute alkaline Ruthenium red.
Deep blue—blue violet with dilute Methylene blue.
Brown or in some cases colorless with chlorzinc iodide.
Unstained with Methyl green unless combined with cutin and cellulose.
Unstained with Magdala red (echt) unless combined with cutin and cellulose.
Insoluble in copper oxide ammonia.
Soluble in hot dilute mineral acids followed by hot dilute ammonia.
Soluble in 50% chromic acid.
Optically isotropic.

Cutin.
Red color with Sudan III.
Red color with Scharlack R.
Violet stain with Methyl green when combined with cellulose and pectic materials.
Colorless with Methyl green when free from cellulose and pectic materials.
Red color with Magdala red (echt) when combined with cellulose and pectic compounds.
Colorless with Magdala red (echt) when free from cellulose and pectic compounds.
Brown color with chlorzinc iodide.
Insoluble in 50% chromic acid, copper oxide ammonia, and hot dilute mineral acids.
Saponified with hot concentrated KOH.
Optically negative when combined with cellulose.
Optically anisotropic when combined with cellulose.
Optically anisotropic or optically isotropic when free from cellulose.

Mention should be made of the importance of Methyl Green and Magdala Red introduced by Kisser (4) as a means of detecting cutinized cellulose. Methyl Green commonly contains small quantities of Methyl Violet as an impurity. This Methyl Violet is strongly absorbed by cutinized cellulose containing pectic compounds from dilute solutions of Methyl Green. Beautiful results may be obtained with the dye if properly used and it can be most instructive in determining the presence of combinations of the three membrane constituents mentioned. The intensity of the stain seems proportional to the abundance of cellulose and pectic materials. Magdala Red works equally well, giving a distinct red color that sharply differentiates the different regions in the wall.

These dyes are also helpful in studying lignification as Kisser has pointed out.

Through the courtesy of Professor W. Seifriz the writer was able to examine preparations of the wall with the Spierer lens. The image gave the characteristic striated appearance reported by Seifriz (6) and others in the cellulose and outer cutinized portions of the wall. The inner zone of cutinized cellulose presented an entirely different aspect. In place of the symmetrically arranged striations the dark rods were chaotically distributed, giving the appearance of much folding and irregularity of arrangement. This indicated a different physical relationship in the inner zone of cutinized cellulose over that prevailing in the outer portions of the wall.

In addition to the interference colors obtained with a gypsum plate Red I, the polarizing microscope permits the use of dichroism as a tool in cell wall work. Ambronn discovered this phenomenon in 1888 but it was the work of Frey-Wyssling (3) that has demonstrated the importance of the phenomenon in cell wall work. Dichroism is apparently the result of the micellar structure of the cellulose wall. When a bast fiber of flax (Linum) is stained with chlorzinc iodide and examined with a polarizing microscope, using the polarizer only, the color of the fiber varies from almost black to colorless when the stage is rotated. The following quotation from Frey-Wyssling indicates the way in which this phenomenon may be utilized in studying the distribution of cutin in epidermal walls:

"Die gelben Reaktionen von Lignin, Suberin und Kutin sind im Gegensatz zur Zellulosereaktion nicht dichroitisch. Frisch gefärbte Schnitte von Holz- und Korkgeweben, sowie stark kutinisierte Epidermen (z. B. von *Clivia nobilis*) weisen keine Intensitätsunterschiede auf, wenn man den Objekttisch über dem Polarisator dreht.

"Diese Feststellung ist wichtig; denn sie gestattet uns den Nachweis von Lignin und Kutin in nur schwach imprägnierten Zellmembranen, die mit vielen Reagentien nur Zellulosereaktionen geben. Färbt man solche Objekte, wie z. B. Jutefasern oder die langen Haare des Fruchtschnabels *Erodium gruinum*, mit Chlorzinkjod und bringt sie über dem Polarisator in die Stellung 'farblos,' so erscheinen sie je nach dem Ligningehalt schwächer oder stärker gelb gefärbt.

"So erlaubt uns das Chlorzinkjod nicht nur völlig verholzte und kutinisierte Membranen zu erkennen, sondern mit Hilfe

des Dichroismus der Zellulosereaktion können in der Stellung des Absorptionsminimums selbst die ersten Anfänge der chemischen Zellwandveränderungen, die oft schwer nachzuweisen sind, spielend enthüllt werden."

All of the above mentioned methods have been used in studying this epidermal wall—and results from the various methods are in agreement.

RESULTS.

Staining the wall with Sudan III brings out clearly the two distinct zones of cutinization (Plate I, Fig. 1). The outer zone and the inner zone of the cutinized wall are distinctly separated by a colorless zone of cellulose and pectic material. The structure of this deposit has been reported in an earlier paper (1). In some cases the inner cutinized zone is adjacent to the protoplasm of the epidermal cell while in other cases a second zone of cellulose separates the protoplast from the inner zone of the cutinized wall. Both conditions may be seen in the figure cited. Careful staining with dilute Sudan III reveals a difference in intensity of stain in the outer portion of the wall. The same effect may be produced by staining with Sudan III and then carefully washing out the stain with alcohol. The vertical boundary line between each cell is emphasized by a deeply staining, vertical, rod-shaped region (Plate I, Fig. 2). This zone differs decidedly from the rest of the cutinized wall in its higher content of cutin, and in the ease with which it may be saponified. The cellulose in the outer wall is not continuous, but grouped in a series of fine horizontal lamellae over each cell. Between these groups of cellulose lamellae extends this narrow vertical partition of nearly pure cutin, partially isolating each cell from other cells in the outer wall. This structure is clearly revealed when the wall is saponified with concentrated KOH (Plate I, Figs. 5 and 6). The presence of this vertical partition of cutin between the epidermal cells is further revealed in striking fashion by the polarizing microscope (Plate I, Figs. 7 and 8). The outermost layer of cutin, the cuticle, is more resistant to saponification and remains as a definite sheet-like layer when the cutin has been removed from the remaining portions of the wall with saponifying agents (Plate I, Figs. 5 and 6).

The presence of cutin in the inner cutinized zone is not only indicated by the adsorption of Sudan III, but also by the action

of concentrated chromic acid. This reagent quickly removes the cellulose but aside from a slight swelling that reveals a distinct lamellation, the inner zone of cutin is unaffected (Plate I, Fig. 4). Apparently this inner cutinized zone consists of a group of parallel lamellae of cellulose, separated by pectic material and infiltrated with cutin. Saponification of the cutin in this zone produces globules of soap as readily as the cutin present in the outer portion of the wall (Plate I, Fig. 5). The lamellae that are visible after saponification give the recognized tests for cellulose. The inner zone shows the interference colors characteristic of cutinized cellulose, though the colors are less conspicuous than those of the outer portions of the wall (Plate I, Fig. 8). The high pectic content of the inner cutinized zone is indicated by the intensity of the staining reactions (Magdala Red, Methyl Green), by the solubility reactions and by the marked decrease in double refraction of this portion of the wall (Plate I, Fig. 7). The inner cutinized zone exhibits a slight but definite double refraction and gives the dichroism characteristic of cutinized cellulose when treated with chlorzinc iodide.

DISCUSSION.

The outer epidermal wall of the leaves of the *Clivia nobilis* plant studied, differs from the outer epidermal wall of most Clivia plants in having two definite zones of cutinized cellulose. The cellulose deposited by the protoplasm of the epidermal cell is unevenly distributed around the vacuole, being much thicker on the outer wall. This cellulose deposit is not homogeneous, but consists of a series of fine horizontal lamellae separated by colloidal pectic material. The amount of pectic material varies in different portions of this outer wall and the present investigation confirms the structure reported in an earlier paper in this respect. This complex of cellulose and pectic lamellae is deposited only above each protoplast and does not extend continuously from cell to cell. The apparent continuity of the epidermal wall is due to the presence of cutin that impregnates the stratified deposition of each cell and fills the spaces between the cells, welding them into one wall. The outer epidermal wall is therefore essentially a mosaic of the cutinized cellulose of individual cells bound together by vertical partitions of cutin.

The inner zone of cutinized cellulose has in general the same

fundamental structure present in the outer portion of the wall. The deposit consists of a series of thin cellulose lamellae, separated by and impregnated with colloidal pectic material. To this has been added the cutin. The thickness of this inner cutinized zone varies widely in different cells of the same section and is in general thicker in the upper epidermis than in the lower. In some cells the deposit is apparently in direct contact with the living protoplast while in others it is separated from the protoplast by a second deposit of cellulose.

Concerning the cause for this unique deposit little can be said. It is not general and the author has not seen it in his earlier work with Clivia plants. It seems limited to relatively few individuals through an examination of the leaves of Clivia plants in various parts of the country may reveal that the habit is more general than realized at present. The deposit is of particular interest in regard to present theories of the mechanism of cutinization. Lee and Priestley (5) have suggested that the fatty constituents of epidermal walls may reach the epidermis by migration along the radial walls of the subepidermal tissues. On reaching the outer wall the fatty materials are condensed and oxidized forming the cutinized portions of the epidermal wall. The present study offers support to their suggestion that exposure to the light and air modifies considerably the character of the fatty deposits for the outermost layer is decidedly more resistant to saponification than are the deposits of cutin within the wall itself. It is difficult to account for this inner localized zone of cutin completely surrounded by cellulose, or at least bordered by cellulose on the *outer* margin on the assumption that the fatty constituents were migrating to the surface through the walls of the subepidermal cells and deposited in the walls through the loss of water.

In this case the epidermal wall seems to be produced by the epidermal cells themselves, and furthermore it indicates that the mechanism of cutinization may be more complex than generally realized at present. It is not impossible that the cutin partitions between the cutinized cellulose of each epidermal cell may arise from subepidermal tissue and reach the epidermis by a migration, and it may be that some of the cutin in the outer wall has a similar origin. The general structure of the wall and the presence of these peculiar localized zones of cutin in the wall do not support this suggestion. It seems probable that in this wall the cutin as well as the cellulose and pectic

materials are all the result of the activities of the epidermal cells alone.

The inner cutinized zone is usually characterized by the appearance of numerous fine granules of fatty material. The delicate cellulose lamellae seem at times to be strings upon which cutinized beads are strung. The cutin does not have the smooth uniformity of that in the outer portions of the wall. These granules may be seen in Figs. 1, 3, and 4 of the plate. The Spierer lens further indicates that the arrangement of the units composing the wall are not regular nor uniform in this inner cutinized zone. The significance of these fine granules is unknown. In some cases gaps and spaces are evident in the inner cutinized zone. These may have resulted from sectioning but seem to indicate that this portion of the wall has less cohesion than other portions.

A large part of the experimental work described in this paper was accomplished in the Plant Microchemical Laboratories of The Ohio State University and the author wishes to express his appreciation of the many courtesies extended to him by members of the Botany Department of that institution. The author further wishes to acknowledge his indebtedness to Professor Seifriz, of the University of Pennsylvania, for the opportunity of examining sections of this wall with the Spierer Lens and to thank Dozent Dr. Frey-Wyssling, of Zürich, for his courtesy in checking dichroic and interference phenomena of the wall.

SUMMARY.

1. The epidermal cell walls of the leaves of some *Clivia nobilis* plants show two distinct zones of cutinization.

2. The inner zone of cutinized wall consists of a series of cellulose lamellae separated by layers of pectic material, both of which are impregnated with cutin.

3. The inner cutinized zone may be in direct contact with the protoplasm of the cell or may be separated from the protoplasm by a second zone of cellulose and pectic materials.

4. The inner cutinized zone is separated from the outer cutinized zone by cellulose and pectic material. The structure of this deposit has been reported in an earlier paper.

5. The cutin is not uniformly deposited in the outer cutinized zone, nor has it the same chemical and physical properties in all portions of the zone.

6. The outermost layer of the wall contains no cellulose

nor pectic material and is more resistant to saponification than other cutinized areas of the wall.

7. The cellulose and pectic material in the outer wall of each epidermal cell are separated from other cells by vertical partitions of cutin that contain little or no cellulose.

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EXPLANATION OF PLATE I.

(All figures refer to cross sections of epidermal cells of leaves of *Clivia nobilis*.)

Fig. 1. Wall stained with Sudan III. Cutinized regions black, cellulose and pectic materials free from cutin are white. 440×

Fig. 2. Wall stained with Sudan III and partially destained with alcohol, showing vertical partitions of cutin between the outer wall of each cell. 440×.

Fig. 3. Wall stained with Magdala Red (echt). Inner zone of cutin, pectic materials and cellulose stains, vertical cutin partitions revealed and accumulation of pectic material indicated at boundary between cellulose and outer zone of cutinized cellulose. 440×.

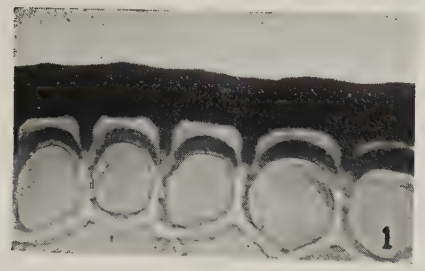
Fig. 4. Wall treated with 50% Chromic acid. Cellulose largely removed. Inner zone of cutinized cellulose swollen to reveal lamellated structure. 440×.

Fig. 5. Wall treated with hot conc. KOH, followed after washing, with Chlorzinc iodide. Resistant cuticle remains. Vertical cutin partitions between cells removed, cellulose lamellae in outer walls stained blue. Inner zone of cutinized cellulose showing lamellations and soap globules. 440×.

Fig. 6. Wall treated with hot conc. KOH. Vertical partition of cutin completely removed. Soap globules apparent in zone of cutinized cellulose. Cuticle intact showing high resistance to saponification. Outer portion of wall only is shown. 440×.

Fig. 7. Wall as seen between crossed Nicols. Pectic zone in the wall appears as a black line. High pectic content of the inner cutinized zone markedly reduces its double refraction. 500×.

Fig. 8. Wall as seen between crossed Nicols with gypsum plate Red I inserted showing Interference colors. Vertical cutin partitions conspicuous. Inner cutinized zone optically negative indicating presence of cutin. 500×.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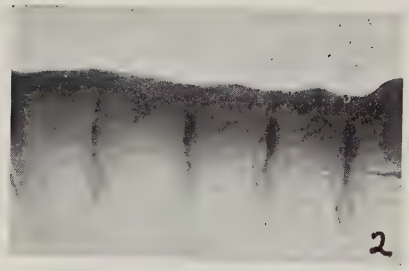
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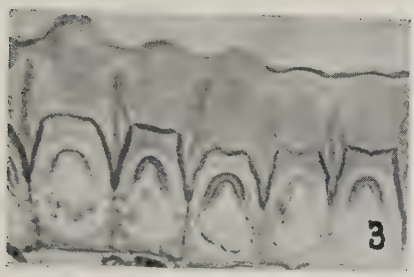
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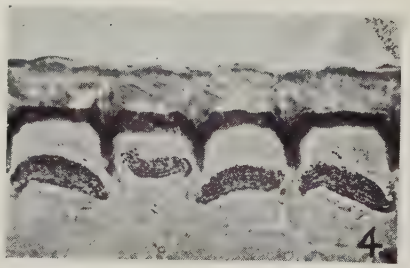
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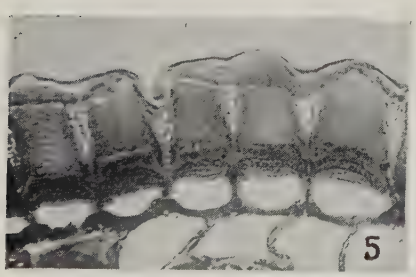
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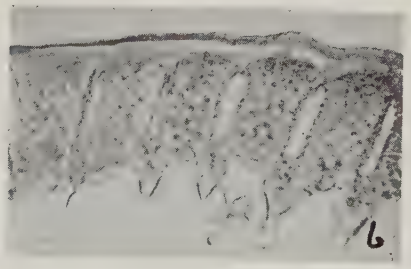
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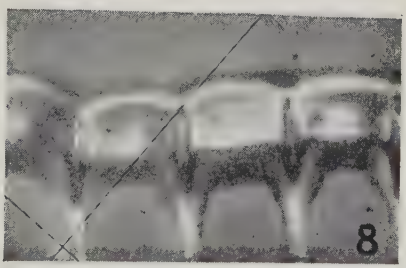
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THE RED-BELLIED WATER SNAKE, NATRIX SIPEDON ERYTHROGASTER (FORSTER), IN OHIO.

ROGER CONANT
Toledo Zoological Society.

During a study of the reptiles indigenous to Ohio four water snakes of the genus *Natrix* have been collected. Three of these, *Natrix sipedon sipedon* (Linné), *Natrix septemvittata* (Say) and *Natrix kirtlandii* (Kennicott) are widely recognized and are known from numerous localities in the state. The fourth, a rarer, large, red-bellied form, has been identified as *Natrix sipedon erythrogaster* (Forster). There seems to be no general agreement among herpetologists as to the validity of the latter which has long been confused with *Natrix sipedon sipedon*. Stejneger and Barbour (1923, p. 108) do not admit it to their "Check List of North American Amphibians and Reptiles," but refer to it in a footnote which reads, "Apparent forms which are more or less uniformly rufescent are found in the ranges of both the subspecies (*Natrix sipedon*) *fasciata* and (*Natrix sipedon*) *sipedon*. To these the names of *Natrix sipedon erythrogaster* and *Natrix sipedon fulviventer* have been given."[1] Blanchard in his key to North American snakes (1925a, pp. 9–10) includes *Natrix sipedon erythrogaster*, but mentions no locality as far north as Ohio. In "The Herpetology of Michigan," by Ruthven, Thompson and Thompson (1912, p. 95) and Ruthven, Thompson and Gaige (1928, pp. 105, 108) *erythrogaster* is not recognized as distinct, although Clark (1903, pp. 1–23) discussed in detail the differences between this form and *sipedon* as seen in a series of specimens from southern Michigan and concluded that it should be considered as a distinct species. Taylor (1929, p. 57–58), writing on the snakes of Kansas, favors a similar interpretation as does also Blanchard in papers (1922, p. 12, and 1924. pp. 537–538) published before the appearance of his key (1925a).

With a view to shedding some additional light on the systematic status, natural history and range of this reptile, in

[1]This foot note is repeated in the third (1933) edition of "A Check List of North American Amphibians and Reptiles," by Stejneger and Barbour.

so far as its occurrence in Ohio is concerned, the red-bellied water snake is here discussed in some detail. Young specimens obtained from captive females are briefly described and compared with the young of *Natrix sipedon sipedon*. A small series of *sipedon* from the same localities in which *erythrogaster* has been collected is considered for comparison as are also certain statistics gleaned from a study of 100 specimens of *sipedon* selected at random from various parts of the state.

While reported from other localities, *erythrogaster* has so far been taken in Ohio in only two places, these, with attendant data, being as follows:

1. Near Blakeslee, Williams County, (60 miles west of Toledo), 11 adult specimens of *erythrogaster*, one adult and one juvenile specimen of *sipedon*.

2. Near Mt. Victory, Hardin County (80 miles south of Toledo), five adult specimens of *erythrogaster*, one of which (No. 1690),[2] collected July 23, 1932, gave birth to eight young September 30, 1932, and one (No. 1662), collected May 29, 1932, had 18 well formed embryos removed from her upon her death October 23, 1932. Two specimens of *sipedon*, one of which (TZS No. 1518) collected July 23, 1932, gave birth to 13 young September 14, 1932.

The only Ohio record in the literature is by Morse (1904, p. 131), who mentions collecting *Natrix fasciata erythrogaster* Shaw at Put-in-Bay, but the only specimen (No. 41) in the collection of the Ohio State Museum identified as *erythrogaster* and bearing Morse's name as collector is definitely *sipedon*. The writer has examined 202 specimens of water snakes from the Ohio Lake Erie islands and the adjacent mainland without finding a single one which approaches *erythrogaster*, although several have a pinkish or light orange mid-ventral surface as do others from various parts of the state and particularly from southern Ohio. A large percentage of the specimens from the islands exhibit peculiar abnormalities of pattern which it is planned to discuss in a longer paper now in preparation.

From the above it will be seen that there is available a total of 42 specimens of *erythrogaster* and 17 of *sipedon* from the Williams County and Hardin County localities. A careful

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[2]All of the known specimens of *erythrogaster* from Ohio are in the collection of the Toledo Zoological Society except No. 443 in the Ohio State Museum and two individuals in the private collection of Dr. Frank N. Blanchard, of the University of Michigan.

study of this material shows that it agrees in practically every detail with Clark's (1903) specimens from near Olivet, Michigan.

The most conspicuous difference between adult Ohio specimens of *sipedon* and *erythrogaster* is in color. In the latter the dorsal surface is uniform black or brownish black with the belly uniform bright red or scarlet, except for the antero-lateral portions of the ventral scutes which are black or blackish. In *sipedon* the upper surface is usually patterned with a mid-dorsal and lateral series of dark blotches, these being subquadrate in form and superimposed upon a paler ground color. In many of the darker specimens, however, the blotches may be more or less confluent. The belly pattern normally consists of dark semicircles on a lighter ground, but these markings are often reduced or are represented by dotted or clouded areas, while in some individuals the belly may tend towards uniform black, never uniform red as in *erythrogaster*. A light midventral area which varies from white to yellow, orange or pink extends from the chin nearly, or quite, to the anal plate in many specimens. This condition has not been seen in *erythrogaster*.

Young specimens of both *sipedon* and *erythrogaster* have a well marked pattern of blotches on a paler ground, but the arrangement of these is different in the two forms. In *erythrogaster* the lateral blotches alternate with those of the dorsal series farther forward than in *sipedon;* in both forms the tendency is for the most anterior dorsal and lateral blotches to unite, forming dark crossbands, but this tendency is much more marked in *sipedon* than in *erythrogaster*. In *erythrogaster*, of the total of 31 to 42 blotches from the head to a point directly above the anal plate, the fusion occurs in from 1 to 9 of the most anterior blotches with an average of 3.7. In the series of 17 *sipedon*, of the 30 to 37 blotches, the anterior 8 to 14, average 10.6, do not alternate. The average for the series of 100 *sipedon* is also 10.6. In several of these latter specimens the blotches are united to form the above mentioned crossbands almost throughout the length of the body, but in one (TZS No. 540), from Geauga Lake, Geauga County, they are alternated forward to the head although otherwise the specimen exhibits typical *sipedon* characters.

The belly in juveniles of both forms is similar to, but paler than, that of the adults.

TABLE I.

VARIATIONS IN OHIO SPECIMENS OF *Natrix sipedon erythrogaster* AND *Natrix sipedon sipedon*.*

CHARACTERISTIC	*Natrix sipedon erythrogaster*		*Natrix sipedon sipedon*			
	1. From Hardin and Williams Counties	No.† 40	1. From *erythrogaster* Localities	No.† 17	2. From Various Ohio Localities	No.† 100
Ventrals ♂	149 to 156, average 151.8	18	138 to 143, average 140.9	8	135 to 149, average 140.6	52
Ventrals ♀	149 to 157, average 153.5	22	141 to 145, average 142.1	9	135 to 149, average 141.7	48
Ventrals ♂ and ♀	149 to 157, average 152.7	40	138 to 145, average 141.5	17	135 to 149, average 141.1	100
Subcaudals ♂	71 to 84, average 76.8	17	70 to 76, average 73.1	8	68 to 78, average 73.3	45
Subcaudals ♀	64 to 74, average 67.4	17	60 to 64, average 62.3	9	57 to 70, average 62.0	42
Subcaudals ♂ and ♀	64 to 84, average 72.1	34	60 to 76, average 67.4	17	57 to 78, average 67.9	87
Ventrals + Subcaudals ♂	223 to 236, average 228.6	17	208 to 218, average 214.0	8	206 to 224, average 213.8	45
Ventrals + Subcaudals ♀	216 to 228, average 221.5	17	203 to 208, average 204.4	9	194 to 217, average 203.7	42
Ventrals + Subcaudals ♂ and ♀	216 to 236, average 225.1	34	203 to 218, average 208.9	17	194 to 224, average 208.9	87
Eye/Head, Adult ♂	20.4% to 23.0%, average 21.9%	6	21.2%	1	19 % to 22.2%, average 20.4%	18
Eye/Head, Adult ♀	21.1% to 25.1%, average 22.5%	8	19.3% to 19.4%, average 19.35%	2	18.1% to 21.0%, average 19.5%	14
Eye/Head, Adult ♂ and ♀	20.4% to 25.1%, average 22.2%	14	19.3% to 21.2%, average 20.0%	3	18.1% to 22.2%, average 20.0%	32
Eye/Head, Juvenile ♂ and ♀	23.8% to 27.2%, average 25.1%	8	19.2% to 22.6%, average 21.6%	14	19.8% to 22.4%, average 21.3%	39
Blotches from Head to Anus	31 to 42, average 35.7	26	30 to 37, average 34.3	17	26 to 43, average 32.7	89
Blotches not Alternated ♂	1 to 9, average 4.4	12	8 to 13, average 10.1	8	1 to 32, average 11.3	38
Blotches not Alternated ♀	1 to 8, average 3.1	14	8 to 14, average 11.0	9	1 to 27, average 9.8	39
Blotches not Alternated ♂ and ♀	1 to 9, average 3.7	26	8 to 14, average 10.6	17	1 to 32, average 10.6	77

*Imperfections in many specimens prevent complete counts of all characters as:
Only specimens with perfect tails are included in the counts involving the number of subcaudals.
Embryonic specimens are not included in the measurements involving the eye.
Specimens having obscure or very irregular markings are not included in the figures concerning the number of blotches.
All juvenile specimens of *sipedon* included in the computations are under 300 mm. in length.
All adult specimens of *sipedon* are 568 mm. or more in length, this being the size of the smallest large *sipedon* from the *erythrogaster* localities.
†The figures in these columns refer to the number of specimens involved in each series of extremes and averages.

In a series of eight newly born young of *erythrogaster* (from specimen No. 1690) the coloration in life of the dorsal and lateral blotches is black on a ground color of orange brown shading from Mars Orange[3] to Sanford's Brown, although this color is paler in the narrow lines separating the adjacent dorsal blotches. Median blotches are three or four scales long and from nine to eleven scales wide. Lateral blotches are two or three scales wide and extend from the edges of the ventrals to the eighth or ninth row of scales. The belly is uniform orange, ranging from Bittersweet Orange to Orange Rufous, except for the anterolateral edges of the ventrals, which are blackish. The labials are the same color as the belly but the sutures between them are darker and are almost black in some cases. There is a patch of white on the chin shields and gulars.

Both forms tend to darken with age. In *sipedon* the blotched pattern usually persists throughout life although occasional large specimens may approach uniform blackness. In *erythrogaster* the juvenile pattern is replaced by the uniform colors of the adult. A freshly shed specimen of the latter 855 mm. in length shows a faint indication of the blotches which can be counted when held in the proper light. The number of blotches, 33, the anterior four of which do not alternate, falls well within the range of variation as shown above in the series of juveniles.

Correlated with the differences of color and pattern are differences in scutellation. The ventral scutes, including the divided anal plate, average 11.2 higher for *erythrogaster* than for *sipedon* from the same localities and 11.6 higher than for the 100 specimens of *sipedon* from various parts of the state (see Table). The extremes for each form are from 149 to 157 and from 135 to 149, respectively; only at 149 do they overlap, which number is seen in two *sipedon* and only one *erythrogaster*.

The number of subcaudals averages somewhat higher in *erythrogaster*. In males there are 71 to 84, average 76.8, while in males of *sipedon* the limits are found to be 68 to 78, average 73.3. The females of *erythrogaster* vary from 64 to 74, average 67.4; of *sipedon* from 57 to 70, average 62. Both sexes considered together range from 64 to 84 in *erythrogaster* with an

[3]Capitalized color names are those of Ridgway's "Color Standards and Color Nomenclature" (1912).

average of 72.1 and from 57 to 78 in *sipedon* with an average of 67.9.

The higher number of scales in *erythrogaster* is best shown by adding the number of subcaudals to the number of ventrals in each form and comparing the results. Thus it will be seen that *erythrogaster* averages 16.2 more than *sipedon* as is shown in the table.

The eye is somewhat larger in *erythrogaster*, this being particularly noticeable in the young. If the diameter of the eye be divided by the length of the head, measured from the tip of the rostral to the posterior tip of one of the parietals, a figure suitable for making comparisons is obtained. In eight juvenile *erythrogaster* the eye ranges from 23.8% to 27.2%, average 25.1%, of the length of the head; in 13 juvenile *sipedon* from 19.2% to 22.6%, average 21.6%. Juveniles in the series of 100 specimens show nearly the same range and average. In adults the eye of *erythrogaster* is found to average 2.2% larger than in *sipedon*.

Aside from the differences evident in pattern, color and scutellation, certain facts concerning habits and habitats indicate further dissimilarity. *Sipedon* occupies a variety of situations and appears to thrive wherever there is a sufficient quantity of water to assure it a food supply and a haven of retreat. *Erythrogaster*, on the other hand, in both Ohio localities, inhabits the environs of small woodland ponds which frequently become dry in midsummer, and in these localities it is much more abundant than *sipedon;* only two of the latter have been taken in each place, while on May 7, 1932, over 30 specimens of the former were seen in Williams County. *Erythrogaster* has been found much farther from water than any of the several hundred *sipedon* which the writer has collected in the state, and one of the Hardin County individuals was encountered fully 200 yards from the nearest pool. It is the writer's impression that *erythrogaster* is more vicious than *sipedon* and that it is more wary and consequently more difficult to capture.

Of the large number seen on May 7th in the Williams County locality several pairs were breeding and the entire colony appeared to be alert and active; one male specimen swam close enough to the writer to be easily seized. The behavior of this individual was unusual but possible explanations might be found in the mating activities or in the fact that the writer

reeked with the musk-like secretion of the caudal glands which had been liberally sprayed on his person by previous captives.

The present Ohio records, with Clark's (1903) record from Olivet, Eaton County, Michigan, extend the known range of *erythrogaster* a considerable distance to the north. According to Blanchard (1925a, p. 10) this form occurs from the "Lowlands of Virginia and the Carolinas, west to Louisiana and north in the Mississippi Valley into southern Illinois." Additional locality records are available from southwestern Indiana and adjacent Kentucky (Blanchard, 1925b, pp. 384–385), but specimens are apparently lacking between this area and northwestern Ohio. This absence might be a result of the intensive cultivation which, with the attendant draining of the land, has doubtless destroyed many habitats suitable for *erythrogaster*. Careful search, however, will probably reveal it in additional localities in Ohio, Indiana and Michigan.

CONCLUSIONS.

1. The data presented show that *Natrix sipedon erythrogaster* (Forster) is entitled to recognition as maintained by Clark, Blanchard and Taylor.

2. A study of Ohio material shows that *erythrogaster* may be readily distinguished by differences in color, pattern, scutellation, size of eye and habitat from *Natrix sipedon sipedon* (Linné), with which it has been confused.

3. The range of *Natrix sipedon erythrogaster* should be extended to include northwestern Ohio and, in view of Clark's (1903), record southern Michigan.

AUTHOR'S NOTE.

Since the above paper was read before the Ohio Academy of Science on April 4, 1933, certain pertinent data have come to hand which it seems advisable to sum up briefly. The Blakeslee locality was revisited April 22, 1933, and twelve specimens of *erythrogaster* were secured, among them the first juvenile to be encountered in the field. This specimen, 388 mm. in length, agrees in detail with the juveniles born to a Hardin County female as described in the paper except that while the blotched pattern is still discernible along the sides, the dorsal surface is nearly uniform blackish brown above and the belly is somewhat paler and more yellowish.

An adult pair was discovered in mating position on this date, while another male lay coiled nearby. Two of the twelve specimens have been preserved; the others are being kept alive.

ACKNOWLEDGMENTS.

The writer wishes to express his indebtedness to the following persons for aid in the preparation of this paper and for assistance rendered by collecting specimens of *erythrogaster* in the field: Dr. Frank N. Blanchard, Howard K. Gloyd, Wm. M. Clay and Arthur Greenhall of the University of Michigan, Karl P. Schmidt of the Field Museum of Natural History, Charles F. Walker of the Ohio State Museum, and Anne Huch Conant, Charles Burris (who first called the writer's attention to *erythrogaster* in Ohio), Clarence L. James, M. K. Murphy, Byron Gardner, Jr., David Delzell, Cecil Murphy, James Kitzmiller, Jr., Leo Higgins Lawrence D. Hiett and Robert Mattlin, all of the Toledo Field Naturalists' Association.

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FIG. 1. (Upper.) Juvenile specimen of *Natrix sipedon erythrogaster* 9 months old and 355 mm. in length. The pattern, while still apparent, is rapidly becoming obsolete. This snake was born in captivity September 30, 1932 to a female (TZS No. 1690), collected near Mt. Victory, Hardin County, Ohio. Photo by Lawrence D. Hiett.

FIG. 2. (Lower.) Adult male *Natrix sipedon erythrogaster* 1106 mm. in length, collected April 22, 1933, near Blakeslee, Williams County, Ohio. Photo by Howard K. Gloyd.

FIG. 3. Habitat of *Natrix sipedon erythrogaster* near Blakeslee, Ohio, April 22, 1933. More than half of the known specimens of this snake from Ohio have been taken in or near this small pond. Photo by William M. Clay.

A MORPHOLOGICAL STUDY OF WORTHINGTON, OHIO.[1]

PAUL CROSS MORRISON
Michigan State College.

Many of the factors affecting the location, development, and future status of any urban center are of a geographic nature. These factors vary with time and place. Leighly states that, "Their combination at a given place and moment of time is probably absolutely unique."[2] Thus, although the results of the following study of Worthington, a suburban village of Columbus, Ohio, are no doubt true in a broad way of suburban towns in general, the primary interest of this paper is the description and explanation of the unique phenomena in the morphology of this particular chorographic unit.

HISTORICAL BACKGROUND.[3]

During the winter of 1801–02, a colonizing association known as the Scioto Company was formed in the states of Massachusetts and Connecticut. The following spring Col. James Kilbourne, one of the members, set out for the Ohio territory in search of suitable land for purchase and settlement. After traveling many miles on foot through the wilderness, he selected 16,000 acres of land on and near the Olentangy River in what is now Sharon township of Franklin County, Ohio.

In "Articles of Association," the members of the company had agreed in writing that two roads should be laid out in this newly purchased tract; one to run north and south, the other east and west. It was further agreed that a village of one hundred and sixty acres, divided into one hundred and sixty lots, should be laid out at the intersection of the roads. The four lots lying on the four central corners were to be reserved as a public square, and one lot each was to be reserved for church and school purposes. The first settlers arrived on the 20th day of October, 1803, and the following May the village of Worthington was laid out as planned.

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[1]The writer wishes to acknowledge the assistance of Dr. Guy-Harold Smith, Department of Geography, Ohio State University, under whose direction this investigation was made.

[2]John B. Leighly. "The Towns of Malardalen in Sweden, A Study in Urban Morphology," *University of California Publications in Geography*, Vol. 3, No. 1, 1928, p. 3.

[3]"*History of Franklin and Pickaway Counties, Ohio,*" Cleveland, 1880, pp. 418–433.

THE FACTOR OF SITE.

The first settlers naturally settled on a site considered the best for the proposed community, a site which met the demands of early Worthington in an admirable way. The retreat of the glacier left the region a till plain in which the drainage system as a rule cut out valleys and glens having steep bluffs rising from narrow flood plains. However, the bluff upon which Worthington was located rose from the east side of the Olentangy with a more gradual slope than usual. Two tributary creeks had by headward erosion formed a saddle which isolated a summit part way up this slope. Worthington's public square and the intersection of its two main streets were placed exactly at the top of this summit. The village was therefore well drained in every direction.

Good drinking water, made accessible by natural springs and driven wells, was to be had from the lenses of gravel common in the glacial till. The location of the community on a west facing bluff heightened the agreeable and invigorating nature of its climate. Nearby level uplands, occupied by intermingling tracts of hardwood forest and open prairie, provided natural resources of value in the establishing and maintaining of the settlement. Along with the rich bottom lands of the Olentangy, these uplands furnished suitable lands for agriculture. The forests supplied timber and fuel, but were not so dense as to dishearten the people or to hinder greatly the development of agriculture and transportation. The former head of Olentangy navigation was at a falls just above the village, and for a number of miles upstream from this point, swift flowing shallows provided tempting mill sites. Communication from the village to the immediate locality was easy.

The relationships between man and environment are dynamic, however, and thus some of the former advantages of Worthington's site are of little present-day importance. The water power sites and the navigability of the Olentangy are no longer of prime significance. The forests have disappeared, but the fertile fields remain. The drainage factor is also still important, and the principal source of water supply is still from artesian wells near the village. The greatest advantage of site at present, however, is that ample room exists for comparatively unrestrained growth of the village. Only the steep west bluff on the opposite side of the Olentangy offers any serious obstruction.

FACTORS OF SITUATION.

The situation of their village probably gave the founders of Worthington little worry, for as yet few settlements existed in Ohio. Conditions of communication forced each of these to be largely self-sufficient, and being so, their location one to the other was not nearly so basic a consideration as was the selection of suitable local sites. Nevertheless, one angle of situation usually considered in these early times was accessibility to a route connecting the frontier with the mother states east of the Appalachians. By way of the Scioto River, Worthington had easy access to the Ohio River, the chief route of interior travel and trade of that time.

In 1812 the city of Columbus was founded as the result of the location there of the State Capital. This would appear to be the critical point in both the history of Worthington and Columbus. Worthington made a strong competitive bid for the capital, but due to a political maneuver lost out at the last moment. Though the site of Columbus was more favorable for the growth of a large city, there is reason to believe that had the capital been established at Worthington, the relations of Columbus and Worthington would be much different today. At all events, the location of the capital at Columbus gave it the impetus which made it the larger city. Proximity to this large and growing city has been the greatest single factor of situation influencing Worthington's morphology.

Columbus naturally became the focus of communication routes. In 1823, the legislature passed an act incorporating a joint stock company for the construction of a Columbus to Sandusky Pike. This road, following the east bluff of the Olentangy, passed through Worthington. It connected with the Scioto Trail and the National Road at Columbus, and soon became a principal route of travel to northern Ohio. Today it is a part of the important Portsmouth, Columbus, Toledo, State Route 23. The main east-west street of Worthington became a part of the Newark to Marysville Pike. Thus Worthington was and is well situated so far as road communication goes. In 1868, the Sandusky Branch of the Cleveland, Columbus, and Cincinnati Railroad was built. It nearly missed Worthington, for in order to avoid the making of a large number of cuts, fills, and bridges, the right of way followed the level interior of the till plain considerably east of the Olentangy. The railroad in so nearly missing

Worthington probably influenced the plan of the town somewhat, as will be shown.

FUNCTIONAL CHANGES AS DEMONSTRATED BY WORTHINGTON.

For a time after its founding, Worthington probably performed a purely rural function. The first settlers entered a virgin territory where they were not constrained by former inhabitants to settle definite spots. Man being normally a gregarious rather than a solitary creature, these pioneers settled in a group. The nature of their settlement was reminiscent of the New England villages from which they had emigrated. Its compact character prepared it for Indian attacks which never materialized, while enough land could be cleared within a radius of say two miles to form a sufficient physical resource for the support of the people. To meet the simple needs of the people, the products of this resource were slightly processed by such embryonic industries as the grist, shoddy, and saw mills. The village common was the dominating influence, for around it revolved the religion, politics, education, commerce, and home life of the village. It was the common ground for exchange of goods, experiences, and ideas. Thus early Worthington furnished for a time an environment of agriculture, local common interests, and all-around living.[4]

Worthington did not for long retain this rural character. Later settlers, forced to take land farther and farther from the rural center, found it more advantageous to live on the land they were working rather than to travel back and forth to the village morning and evening. Thus, although Worthington still acted in a rural capacity for some of its inhabitants, its function as a cross roads service center for the surrounding country became more important. The dominating factor was no longer the common, but the larger agricultural area.

The population of Worthington had increased from 100 in 1804 to 440 in 1840.[5] Broadly speaking it might be said that a population of 100 to 200 represented the era of rural function, while one around 400 represented the era of cross roads function. From 1840 to 1880, with the exception of a minor fluctuation in 1860, the population remained practically stationary. The census of 1860 recorded 349 people. This temporary drop was

[4]Benton Mackaye. *"The New Exploration, A Philosophy of Regional Planning,"* New York, 1928, p. 59.

[5]Population statistics from United States Census Reports.

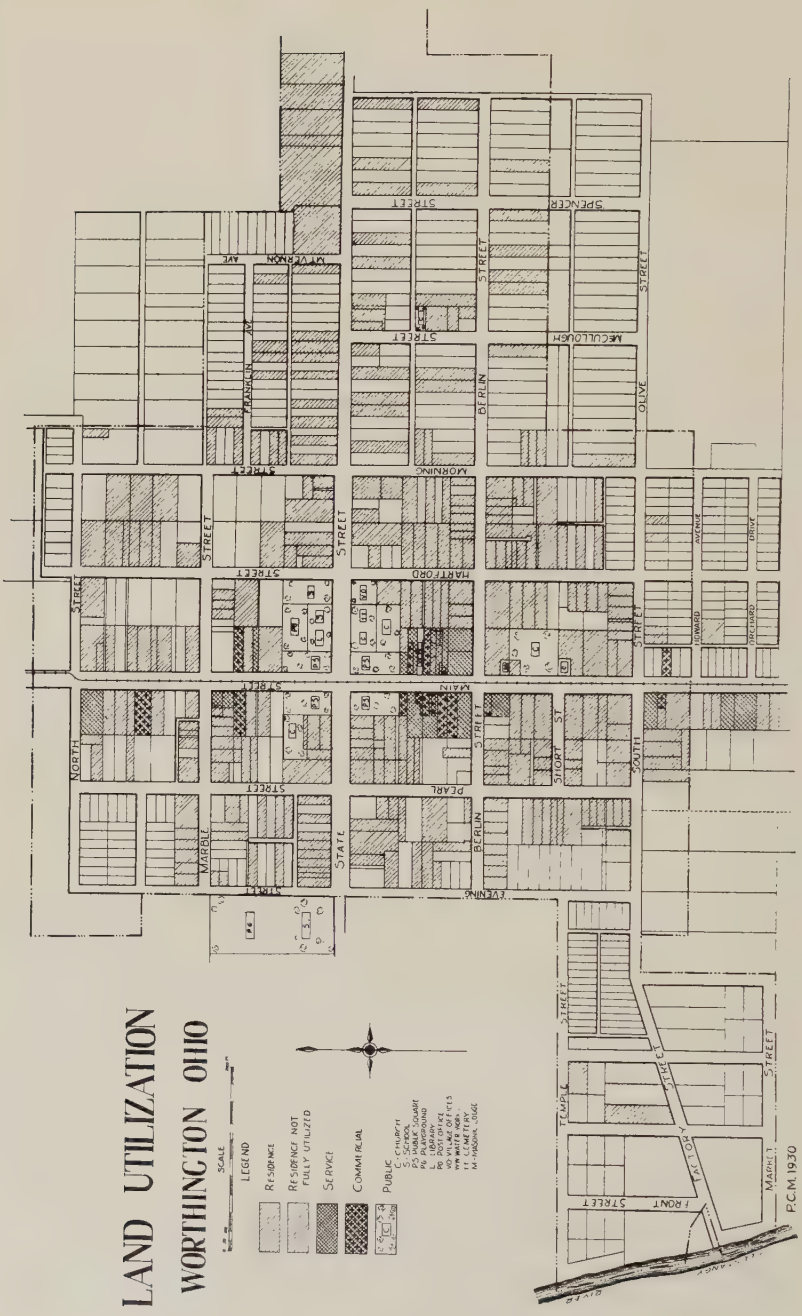
probably due to migration to more western lands. Shortly after 1880, however, the population took a sudden drop and did not again rise above 400 until 1900. In 1890 the population was only 341. It is not at all improbable that the fall in population at this time was caused by the removal of part of the inhabitants to Columbus, for along about 1880 the great industrial consolidations and the consequent growth of banking and insurance facilities caused a wide-spread migration to the cities. Worthington was becoming a drained village so common at that time. Thus for the first time Columbus became the dominating influence in the history of Worthington. The village still retained its cross roads function, but to a greatly lessened degree, however, for with the growth of transportation facilities, the sphere of Columbus influence was greatly enlarged. Many farmers who formerly came to Worthington now went to Columbus to do their marketing and buying.

The sudden migration to the larger cities soon proved somewhat of a boomerang, and a counter flow set in. With development of transportation facilities, people in search of enough ground and air to afford them better living conditions than cound be found in the city, moved to the suburban areas and commuted to their work in the city proper. Worthington was close enough to Columbus to profit by this backwash, and from 1900 to the present time its population has increased. In 1910 it was 547; in 1920, 705; and in 1930, 1,239. During this period Columbus still was the dominating influence, but now in such a way as to change Worthington's predominant function to that of a suburb of the super-urban Columbus.

With the corporate limits of Columbus now within one and one-half miles of those of Worthington, where formerly a space of nine miles separated them, and with both the centers constantly increasing in population and size, it is rather sound theory to forecast their union within a few years. Worthington will have become a submerged village, losing its identity to that of Columbus as a whole. It will then be serving a new function, that of a sub-retail area of Columbus.

GENERAL GROUND PLANS.

The original political pattern of Worthington included one hundred and sixty acres as provided for by the "Articles of Association." This rectangular plot was laid out with its main axis along the north-south road. The first subdivision to be


LAND UTILIZATION
WORTHINGTON OHIO
SCALE
LEGEND
RESIDENCE
RESIDENCE NOT FULLY UTILIZED
SERVICE
COMMERCIAL
PUBLIC
C. CHURCH
S. SCHOOL
LIBRARY
PG PLAYGROUND
PO POST OFFICE
VO VILLAGE OFFICES
W WATER TANK
IT CEMETERY
M MASONIC LODGE
P.C.M. 1930
NORTH
SOUTH
HIGH
STREET
MAIN
STREET
PEARL
STREET
MARBLE
STREET
STATE
STREET
BERLIN
STREET
EVENING
STREET
SHORT
STREET
HARTFORD
STREET
MORNING
STREET
DELAWARE
AVENUE
DRIVE
HOWARD
RICHARD
OLIVE
STREET
MCCULLOUGH
STREET
SPENCER
STREET
DI RUN
STREET
FRANKLIN
AVENUE
WYETHMAN
AVE
TEMPLE
STREET
FACTORY
STREET
FRONT
STREET
MARKET
STREET

added, lay to the east of the original plot where a level continuity of relief afforded satisfactory building sites. The expectancy that these sites would soon be utilized doubtless caused their inclusion in the political unit. Later when the railroad passed slightly to the east of the village in 1868, the desire to have an outlet in this direction probably led to the further extension of the political boundary towards the east. In temperate zones, if no constraints exist to westward expansion, the growth of an urban unit is often westward since the prevailing winds makes the westerly sections more healthful. Two hundred acres just west of Worthington as originally laid out, were reserved for Church and School purposes by the "Articles of Association." This fact has to date no doubt prevented the inclusion of this land within the corporation limits, although the political boundary has been extended to include an area of subdivided land lying to the southwest.

The human occupancy pattern of Worthington, or the areas of land actually occupied by buildings, has assumed the shape of a rude cross which is elongated in an easterly direction. At the intersection of its arms is the public square. The core of the village, a region of low dwelling density, but complete space utilization for business and public purposes, is just to the south. The first settlers naturally built their homes around the public square at the intersection of the two main roads. As more settlers came, expansion was primarily along the more important north-south road to points where the glens of tributary creeks of the Olentangy formed temporary barriers. Growth then took an eastward direction along the main east-west road which gave access to the level upland till plain, joined the original road from Zanesville to Columbus at Newark, and later gave access to the railroad. Expansion in a westerly direction was constrained beyond a certain point by School and Church ownership of the land and by natural restrictions furnished by the Olentangy River. As expansion progressed along these main routes, the intermediate areas were gradually being occupied. The human occupancy pattern has now expanded beyond the glens which partially blocked north-south growth and in the near future it is probable that the greatest growth will be in these directions.

Lands lying in the path of probable directions of human occupancy are usually subdivided long before their use as building sites is demanded. Human occupancy of lands may

take place spontaneously in some unforeseen direction, but no sooner does this happen than lands in that direction are subdivided in expectancy of further expansion. Then again, isolated subdivisions may be established outside of the general forecasted path of expansion with the hope that they can be exploited in such a way as to draw the occupancy pattern in that direction. Worthington's pattern of expectancy of future human occupancy, or the pattern of the various idle or partially idle lands subdivided and held for speculation, though irregular, extends farther to the north and south than in other directions.

MORPHOLOGY OF COMPONENT UNITS: THE LOT.

The lot is the truest and basic component unit of the village, for upon the lots are placed the cultural units which combine to form the village. Worthington was first laid out in rectangular parcels, roughly 134 x 252 feet, or about an acre in area. (See map.). During the first few years when the people lived in the village but gained a living from the outlying fields, the lot on which they lived had to be large enough for the home, a vegetable garden, a barn and other buildings. Land was cheap and plentiful. Thus, the first division of the village land into acre units would seem to be a natural arrangement.

As time went on and Worthington no longer performed a purely rural function, it was not necessary for the lots to be as large as formerly. Land was less plentiful and more valuable as population increased. Since street assessments were based on front footage, it was cheaper to have narrow lots. Thus in the first sub-division just east of the original village, plots 66 x 332 feet were laid out. Another probable reason for the narrow frontage of these lots was the desire of the subdivider to have as many lots as possible facing the main streets. As peripheral expansion takes place, there is also a simultaneous interior expansion. Thus many of the acre lots in the original village were cut up to meet the new conditions.

Still later, as land became even more valuable, complete space utilization was increasingly important. The era of individual self-sufficiency had passed. No longer were vegetable gardens the rule. Village land was becoming too valuable to be used as garden space. Consequently the size of the lot in the most recent sub-divisions averages about 50 x 150 feet. This is large enough for the home, the garden and the small yard.

A glance at the map and the restraining influence of the past

is apparent. Natural property divisions of the past become weaknesses of today.[6] The original acre plots have been broken into lots of various sizes, many of which approach present-day standards. But these acre plots cannot be completely broken up into the smaller lots of today unless additional streets are laid out. This has been done in the reparcelling of the north west square. (See map.) The establishment of more streets would also help to bring the long lots of east Worthington into tune with present day needs.

THE STREETS.

Early Worthington was laid out according to a definite preconceived plan. The streets followed a rectangular pattern. In other words they intersected at right angles and in the case of Worthington ran in true north-south and east-west directions. A street pattern of this nature is easily laid out. It gives straight streets, facilitates location, and makes possible the division of the land into rectangular or square units. One prerequisite, however, is that the physical landscape be fairly uniform.

The two main streets intersecting at the center of the original plot are 99 feet wide. The first streets away from this intersection in every direction are 66 feet wide. The four intersections of the second streets away in every direction form the corners of the original village. These streets are 49½ feet wide. No alleys were provided for in the original plan, and except for private use few have been added. Today this section which represents the original village retains the streets as first laid out, except for the addition of two new streets as the result of changes in the size of lots. Individual land parcels may be split or a building moved, but the alteration of a street to any great degree is rather hard since it has become fixed in regard to all buildings and land units along its length.

In Worthington, planned peripheral expansion has usually anticipated that of a spontaneous nature. The first addition, to the east of the original village, was on fairly level land which permitted the continuation of the general rectangular plan without much difficulty, although it was modified somewhat through the inclusion of more streets as a result of the different shape and size of the lots. Recent subdivisions north and

[6]Robert S. Platt. "Geography of a Sugar District, Mariel, Cuba," *Geogr. Rev.*, Vol. XIX, 1929, p. 604.

south of the village have a planned pattern, but one which illustrates more recent subdivision practices. The pattern is irregular in many places in that streets are laid out to conform with natural features. At other places the streets are curved for purely aesthetic reasons. Thus, as these new subdivisions become a part of the village, the rectangular symmetry of the present street pattern will be destroyed.

THE HOME.

Rude log cabins constructed from the most plentiful and easily used building material at hand satisfied the new settler's immediate need for shelter, but no sooner had the routine life of the village been established than these log huts began to be replaced by the type of home the people had been accustomed to in Massachusetts and Connecticut. The log cabins have thus disappeared, but many of the colonial type homes of the next period are still standing. The most common is the characteristic two-story, rectangular, brick building with a chimney at each end. The long axis of these houses paralleled the street. Often several were built end to end, a customary trait inherited from the east where the earlier homes had been constructed in this manner around the public square as a center and thus presented a solid front to Indian attacks. (Figure 1.)

Following the colonial period came an intermediate period during which the size of homes was increased. (Figure 2.) Homes of several types are representative of this period when style dictated that each house have one or more rooms standing idle a great part of the time, to be used only on special occasions. Building materials were plentiful and comparatively cheap. The houses were either of brick or timber, and were as a rule set back fairly well from the street. Those built during the latter part of the period were more commonly of timber since the extensive exploitation of our forest resources at that time made wood the cheapest building material.

The modern homes are the familiar types of small, fully lived in, well constructed units. These are set near the street, leaving only a small front yard with a fair-sized yard to the rear. Situated sometimes in connection with or near the house, other times at the rear of the lot, and usually attained by means of a front drive from the street, is the garage. This is the only outbuilding customary with the modern home unit. As yet few doubles and only one apartment house exist. The

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FIG. 1. (Upper.) Colonial type buildings. Note end to end construction and characteristic chimneys. This group once served as a tavern, and is now utilized as residences. The building to the right fronts on the public square.

FIG. 2. (Lower left.) One type of residence constructed during the intermediate period. The mansard roof, architectually of French origin, was popular about this time because it afforded an increased top story area.

FIG. 3. (Lower right.) Worthington's only apartment house was formerly a girls dormitory for a now defunct normal school.

FIG. 4. (Upper left.) Part of the retail district. On the right is the oldest standing building in Worthington. At one time part of an inn, later used as a school building and still later as a residence, it today serves as a funeral home. The next building to the left was recently destroyed by fire, and has been replaced by a modern storeroom, the colonial design of which harmonizes with the buildings on either side.

FIG. 5. (Upper right.) A building constructed in 1856 for public school purposes, and used since 1871 as Corporation and Township offices.

FIG. 6. (Lower left.) The oldest church in Worthington; built in 1828. A corner of the larger modern church, recently built on the same lot, is seen to the right. In the background to the left is a school building; in the foreground part of the public square.

FIG. 7. (Lower right.) Built in 1820, the Masonic Hall is a good colonial type building. It is the oldest Masonic building west of the Alleghenies and is said to be the oldest building in the United States that has always been used exclusively for Masonic purposes.

apartment building represents the present utilization of a building formerly used as a dormitory, first for a Methodist Female Seminary, and later for a Normal School replacing it. (Figure 3.)

THE PUBLIC BUILDINGS AND GROUNDS.

Commons were characteristic features of early New England villages, and as already indicated the first settlers carried the idea along to embody it in the structure of Worthington. In spite of the passing of its combined speaking and band stand, the public square still serves as the place of occasional public gatherings. Besides providing for a public square, the village founders provided land for school and church purposes. Coming from a section of the country benefited by good schools, they naturally showed a great interest in education. Almost the first building to be erected was used as a school house. In 1808 the Ohio Legislature passed an act incorporating a school under the name of Worthington Academy, later known as Worthington College, and still later as the Reform Medical School. This last removed to Cincinnati in 1843. The old college buildings were then used to house grade school until they were torn down and replaced by the present public school buildings in 1875.

A preparatory school for Kenyon College at one time occupied the old Kilbourne Hotel, which was situated on the southwest corner of the public square. This school was abandoned in 1863 and the building subsequently served for some time as a residence. The portion still standing today is utilized as a funeral parlor. (Figure 4.) A Methodist Female Seminary, later the Ohio Central Normal School, flourished for a time in Worthington and left its mark on the morphology of the village in the present apartment house.

The first public school building erected after the passage of the State School Law was built about 1856 in the rear of the Episcopal church lot. Its upper story was leased for a term of five years to the Independent Order of Odd Fellows. (Figure 5.) In 1871, upon the removal of the school to the old College buildings, this building was sold to Worthington Corporation and Sharon township and has since been utilized for village offices. At present, in addition to the grade school building on the village school lot, a recently built modern centralized high school occupies the school lands just west of the village.

The oldest standing church building was built in 1828 on the church lot southeast of the public square. Recently replaced by a new building, it is now little used. (Figure 6.) Besides this new St. John's Episcopal Church, the Methodist and Presbyterian denominations both have large modern church homes. Burials in a cemetery established at an early date on the rear of the church lot have long since been discontinued, but many of the old graves still remain.

THE INDUSTRIAL UNIT.

Today not one industrial unit exists in Worthington. Small units such as the tannery, saw mill, grist mill, cooper shop, cabinet shop, and woolen mill, which existed during the era of community self-sufficiency prior to the industrial consolidations of the 1880's, have disappeared. Many of these small industries were housed on the same lot as the home of their owner and operator. They were thus confined to no specific locality in the village. One rather interesting industrial experiment, a forerunner of the modern factory system, took place in early Worthington. In 1811, Col. Kilbourne and others incorporated a stock company under the name of the Worthington Manufacturing Company. A tannery, a shoe shop, a cloth mill, a hat shop, a blacksmith shop and other industries were combined under one roof and one management. This company flourished for a time, having outlets through stores in Worthington, Columbus, and Franklinton, but it finally failed. The factory was located southeast of early Worthington on land now within the corporation limits. A building used as employees' living quarters is still standing.

THE RETAIL BUSINESS UNIT.

Today the retail business district of Worthington is concentrated on High Street just south of the public square. (See map.) Formerly several stores lay north of the square, but most of these have disappeared. Several causes might be advanced for this concentration south of the square. The location of Columbus probably played some part in the drawing of the units in this direction, but this is a rather intangible factor whose importance is hard to determine. Probably a more important factor was that in addition to the buildings originally constructed for store purposes, there existed here several old colonial type homes which, after their days of

residential usefulness, were easily remodeled into inexpensive store rooms. (Figure 4.) Only one or two modern store buildings exist. Then too, the population of Worthington is densest in the southern part of the town and the stores are thus nearer the people. It is interesting to note that only stores providing day by day necessities are found in Worthington. When the inhabitants need something other than groceries, drugs, notions, or hardware goods, they go to nearby Columbus where the larger specialty stores furnish better selections and values. Most of the groceries are sold through chain stores, and it seems to be characteristic of these to locate in close proximity to each other. One type of retail establishment is precluded by its nature from joining in the central concentration. This is the antique shop.

THE SERVICE STATION.

Garages, filling stations, banks, restaurants, barber shops, etc., may be considered as service units. Here in many cases the influence of Worthington's location to Columbus is distinctly evident. It is clear that the people of Worthington alone do not support the five restaurants. These are largely dependent on the patronage of persons driving out from Columbus and upon tourists. The same is true of the garages and filling stations. It is interesting to note that the greatest number of filling stations are on the west side of High Street. This might indicate that they are located thus to be best situated to get the trade of the Columbus motorist entering the village with an empty tank after a long drive in the country, or of the commuter who fills his tank before departing for the city. The service units, other than the filling stations, are concentrated for the most part in the central business district along with the retail stores. It first appears surprising that Worthington has no moving picture house, but with access to the superior play houses of Columbus, the people have little use for a minor neighborhood theatre.

In closing, it might be well to state that although geographic factors explain to a certain extent the morphology of a chorographic unit, they will not entirely do so. However, the geographic approach seems to be justified in the study of the structure and function of urban units.

THE ALIMENTARY TRACT OF PENTHE PIMELIA, FABR. (COLEOPTERA: DACNIDAE).[1]

ROSS W. BRUBAKER
Ohio State University, Columbus, Ohio.

The insect studied, *Penthe pimelia*, Fabr.,[2] is a medium sized (12–14 mm.), broadly oval and depressed beetle of the family Dacnidae. It is gregarious in winter, and hibernates beneath the bark of old logs and old stumps in beech woods. The specimens used in this study were taken in a beech woods near Columbus. The first collection of beetles was preserved during October, 1931, in the following manner: The insects were killed by placing them in water and heating to a temperature of 70° C. (158° F.), then placed in fixing solutions. Half of them were fixed in Kahle's solution; the remainder in Bouin's solution. The mid-intestine was not preserved well enough to permit the cutting away of muscles, trachea, and fat body, without tearing it. The Malpighian tubules were also very brittle. Microscopic sections were made of various parts of the tracts of many different preserved specimens, but most of them were of no value because of the poorly preserved condition of the tissues. It was necessary to obtain live beetles in order to trace the Malpighian tubules in the gross anatomy studies, and to get better material for making microscopic sections.

Sections made from the alimentary tracts taken from live beetles proved to be satisfactory. The digestive juices of this insect act very quickly upon the epithelial lining of the stomach after it is killed, so the beetle was opened under normal saline solution, and when the tract was entirely uncovered, this solution was removed and the fixing agent (Kahle's Fixative, in this case) was put on. Thus the preserving solution reaches the tract and both kills and fixes the tissues before digestion of the stomach lining occurs.

With the exception of the first dozen or so slides made, all sections of the tract were cut at five microns. The usual method of double staining with Haemalum and Fast Green, FCF

[1]Melandryidae of some authors.

[2]The author wishes to express his appreciation for the helpful suggestions and criticisms of Dr. C. H. Kennedy, and for the aid given by Dr. D. M. DeLong in identifying the insect studied.

as described in the laboratory outline for the internal morphology course, was followed for practically all of the slides made. A few were stained with Delafield's Haematoxylin and Eosin. However, the tissues did not stain as well with the latter two, so their use was discontinued.

The drawings were made with the aid of a microprojector.

Gross Anatomy of the Digestive Tract.

GENERAL ANATOMY.

The alimentary canal of *Penthe pimelia* Fabr., is a simple tube extending from the mouth to the anus, with only one loop in its entire length. It is about one and one-half times as long as the insect's body. The three principal divisions—the fore-intestine, the mid-intestine, and the hind-intestine—are easily distinguished in this beetle (Plate I, Fig. 1).

The fore-intestine consists of the mouth, the pharynx, the oesophagus, and terminates at the oesophageal valve.

The mid-intestine is straight and of the same structure throughout its length. It begins at the oesophageal valve, and ends at the pyloric valve.

The hind-intestine begins at the pyloric valve. The Malpighian tubules, six in number, arise at this point. They appear singly and at regular intervals around the circumference of the valve. The ileum is considerably smaller than the stomach, being only about one-fourth the diameter. It goes back from the stomach, turns to the left, under the colon, goes forward into the first abdominal segment, then turns to the right again and goes back to the colon. The colon is somewhat larger than the ileum and a little more than half as long. The distal ends of the Malpighian tubules are attached to the colon by a sheath of connective tissue. The tubules are regularly spaced around the circumference of the colon, each one, however, following a much convoluted path to the caudal end of the colon. The rectum is slightly less than half as long as the colon, and connects the rest of the tract with the anal opening. It is a straight tube, smaller in diameter than the colon, but larger than the ileum.

The size of any part of the canal varies from these proportions when food is present in it.

GROSS STRUCTURE OF THE FORE-INTESTINE.

The fore-intestine is a short tube about one-fifth the length of the body, extending from the mouth back through the head to approximately the middle of the prothorax, where it joins the mid-intestine. It is composed of the pharynx, the oesophagus, the (crop), and terminates at the oesophageal valve.

The *pharynx* is the slightly wider part of the tube just back of the buccal opening. The *oesophagus* is the part of the tube connecting the pharynx to what may be the crop or may be merely a part of the oesophagus which is larger than the rest. This larger portion connects

with the mid-intestine at the *oesophageal valve*, which appears externally as a heavy ring of muscle.

GROSS STRUCTURE OF THE MID-INTESTINE.

The anterior end of the mid-intestine is marked by the muscular oesophageal valve. The mid-intestine varies in size, depending upon the amount of feeding the insect had done at the time it was killed. When full of food material, it is nearly cylindrical, perhaps a trifle larger at the posterior end than farther forward, and occupies about one-half the length of the body. The stomach of a beetle which has not been feeding prior to the time of examination is somewhat contracted, and may be only three-fifths as long as when full. The surface of the mid-intestine is covered with many small projections or crypts. These are of approximately the same size the entire length of the stomach. The posterior end of the mid-intestine is marked by the attachment of the Malpighian tubules and by the pyloric valve.

GROSS STRUCTURE OF THE HIND-INTESTINE.

The hind-intestine is approximately two-thirds as long as the body, and is distinctly differentiated into its various parts, that is: the pyloric valve, the Malpighian tubules, the ileum, the colon, and the rectum.

The *pyloric valve* is evident externally as a heavier walled portion of the canal at the posterior end of the stomach.

The *Malpighian tubules* are six in number and are attached to the canal at the pyloric valve. They are evenly distributed around the circumference of the tract. They are approximately twice as long as the beetle itself, and are much convoluted. The tubules begin at the pyloric valve, wind around in the body cavity in the region of the ileum, and one loop of each goes forward along the mid-intestine, then back to the colon, where it becomes encased in a sheath of connective tissue surrounding this part of the canal. The tubule becomes more or less flattened after it attaches to the colon, and pursues a zig-zag path caudad under this sheath to the point where the rectum begins, and there ends. All the Malpighian tubules are fastened to the colon by this layer of connective tissue or "peritoneum," and are regularly spaced around it. The tubules are colorless and very hard to distinguish for a short distance from the point of origin at the pyloric valve. They gradually become more yellow in color as the distance from the attachment increases. This coloration is pronounced for the last third of the length of the tubules, but disappears when they enter the sheath of tissue surrounding the colon, and for the remainder of their length they are colorless.

The *ileum* is a slender tube leading from the pyloric valve to the colon. As stated in the general discussion of the gross anatomy, the only loop in the main tract occurs in this part.

The *colon* is considerably larger in diameter than the ileum, nearly twice as large, and it is sheathed in a case of connective tissue which holds the Malpighian tubules tightly to it. It appears to be less muscular than the ileum.

The *rectum* is the short straight tube leading from the colon to the anus.

Histological Structure of the Digestive Tract.

HISTOLOGICAL STRUCTURE OF THE FORE-INTESTINE.

The fore-intestine has the same histological makeup throughout its various parts. The tissues present from within outwards are found in the following order Plate I, Fig. 2:

(1) Intima (or chitinous cuticula).
(2) Epithelial layer of cells (Hypodermis).
(3) Basement membrane.
(4) Longitudinal muscle.
(5) Circular muscle.

The innermost lining of the fore-intestine is the *chitinous cuticula* which is continuous with that of the outer body wall, and is secreted by the fore-gut epithelium. The intima is in wave-like folds, and its inner surface is more or less dentate up to the oesophageal valve. At the opening through the valve proper, the *intima* is smooth, and it remains so to where it meets the gastric epithelium.

The *hypodermis* is a layer of irregularly-shaped cells just beneath the intima, and parallels the convolutions of this layer. The *basement membrane* is not clearly shown.

The epithelium is surrounded by a well developed *layer of longitudinal muscles*. These follow the waves of convolutions of the intima to some extent and are more abundant inside of pronounced folds.

The *circular muscle layer* is also well developed. It is around the outside of the longitudinal muscles, and forms a sheath for the rest of the tissues.

Although the oesophagus increases in size as it approaches the oesophageal valve, the relative proportion of the different tissue layers remains the same. It is doubtful that this larger portion has any function other than conduction of food, as it is not large enough to be called a crop, nor heavily enough chitinized to be a gizzard.

The *oesophageal valve* is a fold of the fore-intestine which extends into the mid-intestine, then turns back on the outside of itself to meet the mid-intestine (Plate I, Fig. 3). This valve has four lobes in cross-section. The epithelial cells of the part which extends into the mid-intestine and folds back on itself becomes longer and narrower, finally appearing as a columnar epithelium before the fore-intestine meets the gastric epithelium. The valve is surrounded by a heavy band of circular muscles.

At this point the layers of muscle tissue change their relative position; the circular muscles, which were on the outside in the fore-intestine, are inside the longitudinal muscle layer in the mid-intestine.

HISTOLOGICAL STRUCTURE OF THE MID-INTESTINE.

A histological study of the mid-intestine shows it to be similar in structure throughout its entire length. There are five layers of tissue (Plate I, Fig. 4). Starting from the inside, they are:

(1) Peritrophic membrane.
(2) Epithelium of endodermal tissue.

(3) Basement membrane.
(4) Circular muscles.
(5) Longitudinal muscles.

The peritrophic membrane encloses the food material and protects the delicate epithelial cells from the rough particles.

The cells of the epithelial layer vary in structure or shape according to whether they are in the secretory or resting stage. Soon after the insect takes in food, the cells become distended and some of them break off to supply digestive fluids (holocrine secretion) (Plate I, Fig. 4). These cells are replaced by new cells formed by certain regenerative tissues contained in crypts (Plate I, Fig. 5). In the outer end of these crypts are the masses of regenerative tissue called nidi. Externally the crypts appear as papillae which cover the surface of the mid-intestine. They all have the same structure.

The circular muscles appear just outside the basement membrane of the gastric epithelium.

The longitudinal muscles, usually only a thin layer, occur outside the layer of circular muscles.

HISTOLOGICAL STRUCTURE OF THE HIND-INTESTINE.

The pyloric valve is formed by the extension of a fold of the hind-intestine into the lumen of the mid-intestine (Plate II, Fig. 7). This fold is composed of epithelial cells of the hind-intestine, but instead of being more or less cuboid in shape, they become elongated into narrow, closely packed columnar cells. These are lined on the inside with a layer of chitin armed with spines. These spines persist into the ileum for approximately one-seventh of its length.

The function of these spines is unknown to the writer.

The fact that the walls of the tract are noticeably heavier at the pyloric valve leads one to believe that there is probably a heavy ring of muscles there. However, in this insect it is not so. The thickness is due to the columnar structure of the epithelium of the hind-intestine, where it extends as a fold into the lumen of the mid-intestine (Plate II, Fig. 6). In fact, there are very few muscles of any kind right at the pyloric valve. The well developed muscle layers of the ileum appear just posterior to the valve.

The Malpighian tubules arise as invaginations of the hind-intestine, posterior to the pyloric valve. The hypodermal cells at the beginning of the tube are columnar, as are those forming the valve, but they gradually assume a more cuboid shape further out from their origin. The chitinous lining can be traced only for a short distance along the invagination forming the tubule. A few spines are also found along this invagination. A cross-section of a Malpighian tubule reveals that the epithelial cells in it are irregular in shape and have large nuclei (Plate II, Fig. 8).

The ileum has all the principal layers of tissue usually found in the hind-intestine. The tissues present, from within out, are:

(1) Intima.
(2) Epithelial layer.
(3) Basement membrane.

(4) Inner circular muscle layer.
(5) Longitudinal muscle layer.
(6) An outer circular muscle layer.

As mentioned above, the intima is provided with spines from the pyloric valve for a short distance into the ileum, approximately one-seventh of the total length of the ileum (Plate II, Fig. 9).

About half way from the pyloric valve to the colon, the waves or folds of the intima begin to appear as six more or less distinct projections. This becomes more and more pronounced, and by the time the colon is reached there are six well marked divisions (Plate II, Fig. 10).

The epithelial cells are more or less irregular in shape and vary in size to some extent, increasing slightly in size as they leave the pyloric valve region. By the time the colon is reached, these cells are comparatively large and more distinct.

The basement membrane is visible only in a few of the sections examined.

The inner circular muscle layer is well developed the entire length of the ileum.

The longitudinal muscle layer is well developed for a short distance posterior to the pyloric valve, then gradually dwindles to a few scattering muscles where the spines on the intima disappear. Approaching the colon, the longitudinal muscles develop into six well marked bundles (Plate II, Fig. 10).

The outer circular muscles are well developed also for the same short distance as the spines on the intima, but they diminish in number and finally disappear at the same level as the spines.

The colon is thinner walled than the ileum, and has the following layers of tissue from within out:

(1) Intima.
(2) Epithelial layer.
(3) Basement membrane.
(4) Circular muscles (inner).
(5) Longitudinal muscles.
(6) A sheath of connective tissue or "peritoneum" which encases the distal ends of the Malpighian tubules between it and the colon proper.

The intima in the colon is smooth. Its wave-like folds nearly fill the lumen at the anterior end of the colon, but recede somewhat toward the caudal extremity.

The epithelial cells are comparatively large and distinct. This layer of cells follows the intima closely.

The basement membrane is apparent in some sections.

The circular muscle layer is distinct, and forms the thin wall of the colon. It is composed of three layers of muscle cells.

The longitudinal muscles are present in six well developed bundles, each situated at the bottom of the division between the six folds of epithelium in intima.

The "peritoneum" or connective tissue is a delicate layer of thin nucleated cells surrounding the Malpighian tubules and the colon (Plate II, Fig. 11).

In some sections there appeared to be tiny canal-like openings leading from the caudal ends of the Malpighian tubules to the heavy chitin lining the tract at the junction of the colon and rectum. B. J. Landis was the first to call attention to this fact after making studies of the digestive tracts of several species of beetles having the tubules attached to the colon as they are in *P. pimelia.*

The rectum has much thicker walls than the colon (Plate II, Fig. 12). The layers of tissue present are:

(1) Intima.
(2) Epithelium.
(3) Basement membrane (occasionally apparent).
(4) Circular muscle.
(5) A very few strands of longitudinal muscle.

The intima is smooth and is in six folds or pads. If the tract happens to be full of food material, the folds are flattened out into six pads. These begin to extend farther out into the lumen as the caudal extremity is approached, and practically fill all the available space by the time the anus is reached. It is the opinion of the writer that these six pads, while similar to the rectal glands described by others, do not function as glands to any great extent, since their shape (including the structure of the cells) seems to vary directly with the presence or absence of food material in the tract. If they were distended when the tract is full of material, it would seem more likely that they might function as glands. However, they are largest when the tract is empty, and are reduced to a very flat layer when the rectum is full.

The cells of the hypodermal epithelium are slightly smaller and narrower than in the colon, and the cell walls are hard to distinguish.

The basement membrane can be demonstrated occasionally in this part of the tract.

The circular muscle layer is very well developed here. It is composed of six or seven layers of muscle fibers.

The longitudinal muscle layer is present as a very few scattered strands.

SUMMARY.

The alimentary tract of *Penthe pimelia*, Fabricius, is divided into three primary regions, namely: the fore-intestine, the mid-intestine, and the hind-intestine. These regions are more or less differentiated in themselves, showing the following parts:

Fore-intestine—The pharynx, oesophagus, and oesophageal valve.

Mid-intestine—Entirely stomach.

Hind-intestine—The pyloric valve, the Malpighian tubules, the ileum, the colon, the rectum.

The layers of tissue present in the different primary regions, from within out, are:

Fore-intestine—Intima, epithelium, longitudinal muscle, and circular muscle.

Mid-intestine—Gastric epithelium, circular muscle, and longitudinal muscle.

Hind-intestine—Intima, epithelium, basement membrane, inner circular muscle, longitudinal muscle, outer circular muscle, and "peritoneum."

The Malpighian tubules are six in number, and arise posterior to the pyloric valve at equal distances around the circumference of the tract. They are approximately twice as long as the body of the insect. One loop of each tubule goes forward along the mid-intestine for some distance (different tubules extend different distances) then go back to the colon, where they are attached to it by a thin membrane of connective tissue called the "peritoneum." Each tubule attaches separately and follows a zig-zag path caudad on the colon to the junction of this and the rectum, where it ends.

Histological examination of the ileum shows an interesting correlation between the presence of spines on the intima of this division for approximately the first one-seventh of its length, and the presence of well developed layers of longitudinal muscle and outer circular muscle, which also gradually disappear as the spines do. Sections posterior to this point show no spines, only a very few scattered longitudinal muscles, and no outer circular muscle layer.

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EXPLANATION OF PLATES.

Plate I.

Fig. 1. Dorsal view of alimentary canal.
Fig. 2. Cross-section of oesophagus.
Fig. 3. Longitudinal section of oesophageal valve.
Fig. 4. Cross-section of mid-intestine.
Fig. 5. Portion of cross-section through mid-intestine enlarged to show details.

Plate II.

Fig. 6. Cross-section of pyloric valve showing Malpighian tubule attachment.
Fig. 7. Longitudinal section of pyloric valve showing spines and Malpighian tubule attachment.
Fig. 8. Cross-section of a Malpighian tubule.
Fig. 9. Cross-section of ileum just posterior to the pyloric valve showing outer circular muscle, longitudinal muscle, and spines.
Fig. 10. Cross-section of ileum near colon showing the development of six folds of epithelium and six bundles of longitudinal muscle.
Fig. 11. Cross-section of colon showing connective tissue surrounding the Malpighian tubules and the colon.
Fig. 12. Cross-section of rectum (when empty).

KEY TO ABBREVIATIONS.

B. MEM....Basement membrane.
COL.....Colon.
C. M.....Circular muscle.
C. M. I.....Inner circular muscle.
C. M. O....Outer circular muscle.
CR.....Crypt.
C. TIS.....Connective tissue or "peritoneum."
E. EPI.....Endodermal epithelium.
EPI.....Epithelium.
F. G.....Fore-gut.
H. EPI.....Hypodermal epithelium.
H. I.....Hind-intestine.
H. SEC.....Holocrine secretion.
IL.....Ileum.
INT.....Intima.
L. M.....Longitudinal muscle.
LU. MG....Lumen of mid-gut.
M. I.....Mid-intestine.
M. T.....Malpighian tubule.
M. T. ATT..Malpighian tubule attachment.
N.....Nucleus.
NID.....Nidus.
OES.....Oesophagus.
OES. V.....Oesophageal valve.
PH.....Pharynx.
P. MEM....Peritropic membrane.
P. V.....Pyloric valve.
REC.....Rectum.
SP.....Spines.

Penthe pimelia, Fabr.
Ross W. Brubaker

PLATE I.

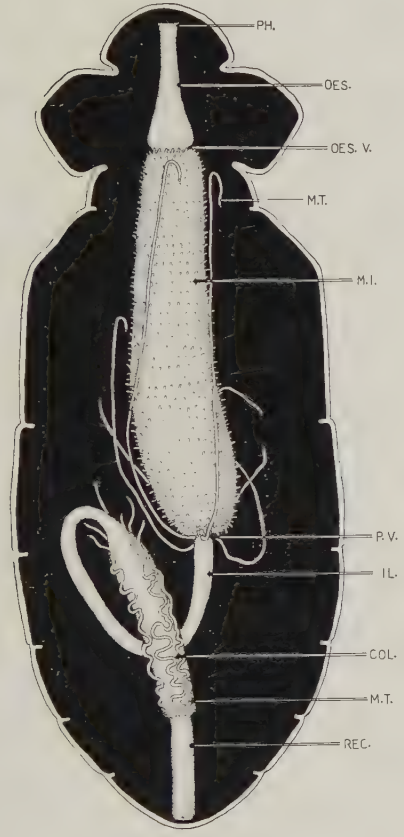


FIG. 1.

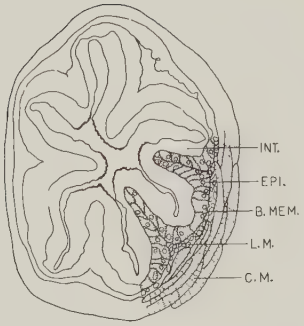
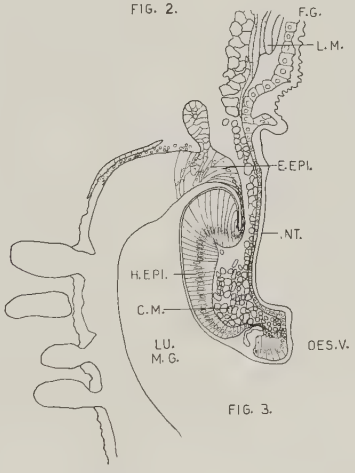


FIG. 2.



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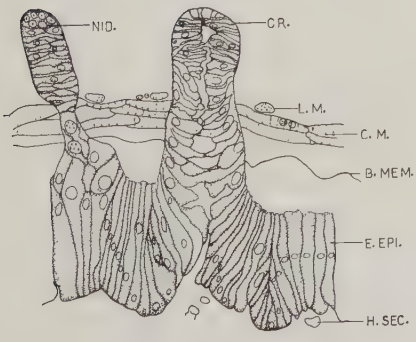

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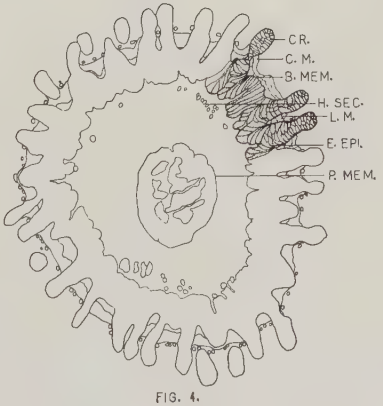
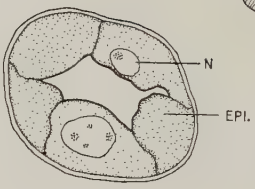


FIG. 4.

FIG. 6.



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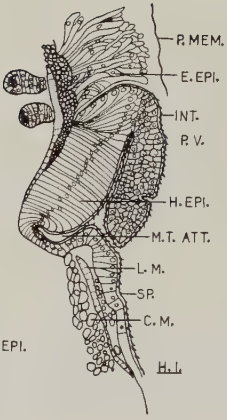
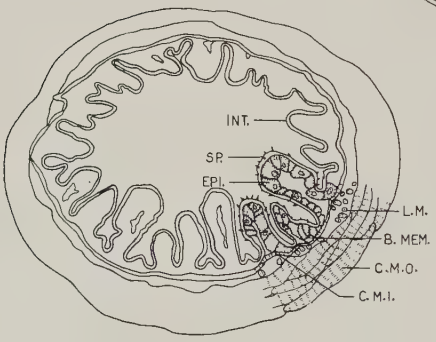


FIG. 7.



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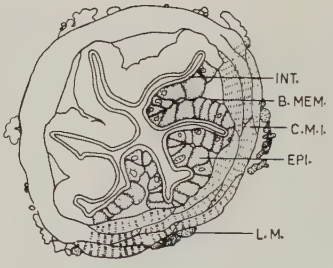

FIG. 10.

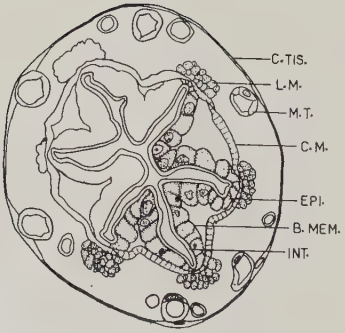

FIG. 11.

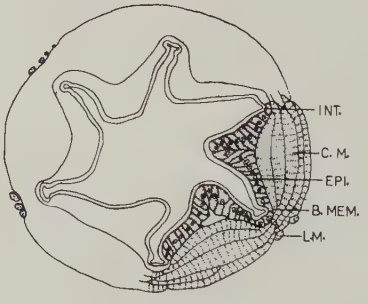

FIG. 12

STUDIES IN THE BIOLOGY OF THE LEECH. II.

Responses of the Leech to Electrical Stimulation.

JOHN A. MILLER,
Ohio State University.

INTRODUCTION.

Direct current of low voltage has proven to be an excellent stimulus of motor neurons when the animal can be completely immersed in water. By this means it is possible to effect a direct motor neuron stimulus or motor neuron depression in those neurons which lie parallel to the current. The direct current acts as a constant force which results in the production of a prolonged response. (Loeb, Lillie, Shensa and Barrows.) It provides a uniform stimulus, the intensity of which may be changed at the will of the investigator to best serve his needs.

The following group of experiments will illustrate the effectiveness of this type of electrical stimulation.

ANTERIOR POSTERIOR STIMULATION.

The leech[1] to be used in this experiment was placed in a glass tray the bottom of which was kept moist. Movable copper electrodes were placed in the tray in contact with the moist surface of the tray. A direct current of 18 volts was used. A reversing switch was connected in the circuit.

The leech was so arranged in the tray that the positive electrode was behind the posterior end of the animal and the negative electrode was in front of the anterior end. When the current was turned on the leech extended to a length surpassing that of normal extension. It maintained this prolonged extension and crawled toward the negative electrode.

When the poles were reversed, that is when the positive electrode was in front of the anterior end and the negative electrode behind the posterior end the leech contracted, and irregular movements of the anterior and posterior ends were pronounced. The anterior end often raised itself from the surface of the container. Following random movements, of the

[1]The leeches used in these experiments were all *Haemopis marmoratis* (Say).

above described nature, the anterior end turned and crawled toward the negative electrode.

Direct current, 18 volts.
The normal length of the leech extended.....14 cm.
The normal length of the leech contracted..... 5 cm.
The average length of the leech..... 7 cm.
With the negative pole at the anterior end the leech extends to a length of.....16 cm.
With the negative pole at the posterior end the length of the leech is..... 7 cm.

A similar experiment to the one above was conducted on the earthworm. The results in both cases were identical. Both worms elongated when the negative electrode was placed in front of the anterior end and both shortened when the negative electrode was placed behind the posterior end.

An explanation of this phenomenon will be given in a later issue of this Journal.

DORSAL VENTRAL STIMULATION.

A zinc plate was placed in the bottom of a shallow glass container. This container was filled with tap water. Near the surface of the water was fastened a galvanized iron screen. This screen was placed an inch to an inch and one-half above the zinc plate. A reversing switch was connected in the circuit. Three dry cells supplied the current.

A leech was placed in the container and came to rest on the zinc plate. With the positive charge on the zinc plate and the negative charge on the wire screen the following reaction occurred:

The leech remained with its mid-ventral surface in contact with the zinc plate. The anterior and posterior ends of the leech were raised above the surface of the plate extending toward the wire screen. The middle region of the leech was contracted. The anterior and posterior ends were extended.

When the poles were reversed, i. e., the negative current connected to the zinc plate and the positive connected to the wire screen, the following reaction occurred:

The leech turned over bringing its dorsal surface in contact with the zinc plate. The leech may roll to right or left, even turning completely over, but eventually it comes to rest with the ventral surface toward the wire screen (positive electrode).

The anterior and posterior ends remain in contact with the zinc plate (negative).

When an earthworm was placed under circumstances similar to that of the leech in the preceding experiment, a somewhat different response was noted. In the first place there did not seem to be a definite orientation to the current. The earthworm rolled over and over, contracting violently at times. This difference in behavior may be explained on the basis of the difference in the regions of concentration of the subepidermal plexus in the two animals. Recent reports from the behavior laboratory (unpublished) give for the earthworm lateral concentrations of the subepidermal plexus. When this mechanism in the earthworm is compared with that in the leech there is some basis for explaining the differences in behavior. As stated in a previous paper there are four concentrations of the subepidermal plexus in the leech, two dorso-lateral and two ventral-lateral. (Miller, 1933.)

The response to dorso-ventral electrical stimulation of the leech was a direct orientation of the animal. The negative current above the dorsal surface directly stimulated the dorsal longitudinal muscle fibers through the subepidermal plexus. The ventral series of longitudinal muscles were inhibited. Through direct motor stimulation in the anterior and posterior ends the circular muscles at the extremities were contracted. This resulted in the leech assuming a U-shaped position.

With the ventral surface of the leech in contact with the zinc plate to which had been attached the negative electrode the longitudinal series of ventral muscles contracted. This contraction resulted in the animal assuming the temporary position like that of an inverted "U." As the leech rolled over, bringing the dorsal surface in contact with the zinc plate, the dorsal series of longitudinal muscles contracted raising the center of the leech toward the wire screen. Throughout this experiment the dorsal surface of the leech has been oriented toward the negative. The anterior and posterior ends had been extended toward the negative. This orientation is the result of direct neuro-motor stimulation on the muscles of the body wall through the subepidermal plexus.

Lateral Stimulation

The bottom of a shallow glass tray was covered with tap water. In this tray was placed a leech. The positive electrode

was placed to the right of the mid-body region. The negative electrode was placed in a similar position on the opposite side of the animal. These electrodes were placed several inches from the edge of the leech and in contact with the water in the container.

Upon the application of the current the leech twisted and rolled. Very shortly after the initial shock the anterior end turned toward the negative pole. The posterior end raised from the surface twisted and turned toward the negative pole. When the poles were reversed the same reaction occurred on the opposite side.

A similar phenomenon to that described above has been known for some time to take place in the earthworm. Of the explanations that have been advanced to account for this behavior, that of Shensa and Barrows (1932) is the most reasonable to date.

The following experiment conducted with leech material is essentially the same as that described by Shensa and Barrows for the earthworm. In this report I will not repeat details of the experiment, as these have been previously reported by the above authors. I will, however, briefly explain the principle of the experiment, the results and the application to the explanation of the leech behavior.

The purpose of this experiment was to determine the relationship between the subepidermal nerve plexus and the lateral muscular contraction of the leech following lateral electrical stimulation.

A strip of the lateral body wall of the leech was removed. This strip of tissue consisted of the epidermis and muscles of the circular and longitudinal series, but did not contain any part of the ventral nerve cord. This strip was suspended in a tumbler of tap water. On each side of the strip were suspended parallel strips of copper screening which served as electrodes. The lower end of the strip of tissue was fastened to a stationary arm, the upper end to a movable writing arm of a kymograph lever. A lever registering time in seconds and another to indicate the current change were placed against the smoked paper of the kymograph drum.

With the negative current applied on the skin side and the positive current on the muscle side, the strip of tissue contracted. Reversing the poles of the electrodes the strip of tissue showed a gradual relaxation.

The same strip of tissue was treated with novocaine after which it was attached and stimulated as before. No muscular contraction could be detected. The same narcotised strip when stimulated directly, that is by attaching a fine copper wire to both ends, responded by definite contraction.

I have shown in the above experiment that the subepidermal nerve plexus exercises the same control over the circular and longitudinal muscles as previously demonstrated for the earthworm, and that this phenomenon explains the behavior of the leech to lateral electrical stimulation.[2] It gives definite evidence of the role played by the subepidermal nerve plexus in annelid behavior.

SUMMARY.

1. Anterior, posterior, and lateral electrical stimulation of the leech and earthworm resulted in identical reactions.

2. Dorsal ventral electrical stimulation of the leech and the earthworm resulted in quite different responses.

3. These differences in behavior are explained on the basis of neuro-muscular differences between the two types of animals.

4. Dorsal ventral electrical stimulation results in a definite orientation in the leech.

5. This orientation is the result of direct motor stimulation (i. e., without intervention of neurons in the nerve cord) on the muscles of the body wall through the subepidermal nerve plexus.

6. Experimental evidence obtained here substantiates the postulated polarity of motor neurons in the leech.

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[2]A detailed explanation of the influence of electrical stimulation and an explanation of certain phases of leech behavior will appear in a later issue of this Journal.

THE CENTENNIAL CELEBRATION OF THE COLLEGE OF MEDICINE OF THE OHIO STATE UNIVERSITY.

On March first, second and third, the one hundredth anniversary of the College of Medicine of the Ohio State University will be celebrated. The college is a direct descendent of the Willoughby College of Medicine, founded at Willoughby, Ohio, in 1834. This college later moved to Columbus, still later changed its names to Starling Medical College, and eventually was incorporated into the Ohio State University.

A comprehensive and appropriate program has been arranged by the committee under the chairmanship of Dr. Ernest Scott. The morning of March first will be given over to clinics by the staffs of St. Francis and the University Hospitals. In the afternoon there will be demonstrations by the laboratories in Hamilton and Kinsman Halls. A banquet in the evening will be followed by a historical program in the Chapel, at which the speakers will be Dr. Packard, the Editor of the Annals of Medical History, Dr. Forman of Columbus, and Dr. Rightmire, the President of the University. Dean Upham will preside.

On March the second at 10:30 A. M. there will be held a special convocation, with all the appropriate academic dignity, at which Dr. Henry S. Houghton will deliver the address. Presentation of honorary degrees will be made to several outstanding medical men. At this convocation will be delegates from various medical colleges, learned societies, and other institutions.

In the afternoon of March second, addresses will be made by Dr. Francis Carter Wood on Cancer, Dr. Edward Francis on Tuberculosis, and Dr. William S. McCann on Metabolism. On this day the class reunions and the fraternity banquets will take place.

On March third, there will be medical and surgical clinics held by specialists from out of the state, and in the evening a public address by Dr. Charles Emerson sponsored by Alpha Omega Alpha.

All alumni and friends of the College of Medicine are invited to attend these ceremonies.

L. H. S.

BOOK NOTICES

Biometric Principles.

This is an excellent introduction to the principles of biometric analysis. Proceeding on the admirable assumption that biologists need a conviction of the usefulness of biometric formulae and a knowledge of the fundamental assumptions involved rather than a mathematician's detailed training, the author develops logically the underlying principles of biometry. "Normal" curves and their statistics, tests for goodness of fit, correlations, predictions, and errors of sampling are all discussed in a lucid and practicable manner. It is to be hoped that Part II of this work will be speedily available.—L. H. S.

Outlines of Biometric Analysis, Part I, by Alan E. Treloar. 65 pp. (mimeograph). Minneapolis, The Burgess Publishing Co., 1933. $1.65.

Great Men of Science.

It is not often that a historical biography of scientists is of a truly readable nature, even to other scientists. The material is apt to be either tiresomely technical, or else to consist of useless and superficial personal idiosyncrasies. This book, however, may lay claim to being a masterpiece. The story of the development of the sciences and of the men who made the development possible is strongly and consistently woven. The book will repay careful reading, and the reading will not be a chore.—L. H. S.

Great Men of Science, by Philipp Lenard. xxii+389 pp. New York, The Macmillan Company, 1933.

British Economic Grasses.

An interesting volume containing keys to 57 British economic grasses. Two keys are presented, one based on the external vegetative structures, the other on the anatomy of the leaf blade and sheath. Of the 57 forms dealt with, 49 are reasonably abundant in eastern North America, many of them being the common grasses of our lawns and pastures. Since the keys in the ordinary manuals are based on reproductive structures, this volume would be valuable to anyone attempting to identify the grasses of lawns and closely grazed pasture lands.

W. H. Camp.

British Economic Grasses, by Sidney Burr and Dorothy M. Turner. 94 pp., 111 figs. New York, Longmans, Green & Co. 1934. $3.75.

Animal Biology.

Professor Wolcott of Nebraska has produced here a textbook which deals with a wider range of subjects than either Zoology textbooks which review a series of type forms, or the typical "biology" text which fails to satisfy the requirements for a standard college zoology course. It is a modification of these two types in which the subject is developed in a logical way, presented in five parts, which contribute to the adaptability of the book.

The author has made a definite attempt in Part I to state and illustrate the general principles and their applications within this field. The body of the text is concerned with a review of the animal groups, in which much more than usual is included on life histories, behavior and environmental relationships. The last fourth of the book deals with general considerations of ecology, evolution, and a whole series of related problems.

The text is well illustrated with many original drawings. The style is clear, forceful and concise. Many common misconceptions are stated as such and ruled out. Behavior is placed on a stimulus-response basis. Statements are unusually if not entirely free from teleology. Above all, the student is made to feel that the animals being discussed are alive. A useful glossary of terms precedes the index.

This book should receive the attention of those presenting general survey courses as a text which presents a wide range of material in a clear, dynamic way, understandable to the student with normal preparation.—JOHN W. PRICE.

Animal Biology, by Rob't H. Wolcott. xvii+615 pp., 335 fig. New York, McGraw-Hill Book Co., Inc., 1933. $3.50.

Hunting Dinosaurs.

These two books belong together, as the second is a sequel to the first. They are the autobiographical account of the author's experiences as a collector of fossils. The man, Charles H. Sternberg, started life in 1850 in New York, moved West in 1865 when his father became principal of a College in Iowa, and two years later went to his brother's ranch in Kansas. Although he was lamed in a youthful accident he early became interested in the collecting of fossils and minerals. This interest became his one aim in life when at the age of seventeen he decided (much to the disgust of his father) to devote himself to that pursuit. His first serious collecting was of fossil plants from the Dakota group. From this he "graduated" into collecting fossil vertebrates, for the most part. The years between 1867 and 1907 brought him into personal contact with such leaders as Leo Lesquereux, the paleobotanist, E. D. Cope, Karl von Zittel, S. W. Williston, Henry Fairfield Osborn, and the great rank of vertebrate paleontologists of that time. He collected for and with them, covering most of the West, visiting known collecting areas or discovering new ones. These contacts enliven the books and give insights into these men. The second book covers his experiences from 1909 to 1917 during which time he was collector and chief preparator for the Geological Survey of Canada. Since then he has "retired" to California although still actively collecting. (I had a letter from him this past spring offering fossils for sale.)

We often read with pleasure the life of a game hunter. Let us try the life of a really **big** game hunter. Here is an opportunity to obtain a better insight into the trials and tribulations of hunting fossil "big game." Now and then the author diverges from the course of events and draws careful and detailed "word pictures" of scenes from out of the geologic past, clothing the land with plants and filling the sea, air, or woods with the proper animals. The books leave one with a renewed wonder at what has passed and a great respect for one of the oldest and earliest collectors. His collections are now found in most of the great museums of the world.

The chronological order of events is not strictly followed, and though jumping backwards and forwards in time is confusing. The illustrations are all halftones and give the reader an idea of the animals mentioned and an idea of the difficulties encountered in collecting, especially in the earlier years of the work.

WILLARD BERRY.

Life of a Fossil Hunter, by Charles H. Sternberg. 286 pp. San Diego, 1931. $1.75.

Hunting Dinosaurs on Red Deer River, by Charles H. Sternberg. 261 pp. 1932. $1.75. (Published by Charles H. Sternberg, 4046 Arizona St., San Diego, California).

Sixth International Botanical Congress.

Those interested in the botanical sciences are requested to note that the time of the Sixth International Botanical Congress has been changed. The Congress will meet at Amsterdam, Holland, from September 2nd to September 7th, 1935.